VOLUME 77 *January 1958* NUMBER 1

THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

OFFICIAL JOURNAL OF AMERICAN TRUDEAU SOCIETY
MEDICAL SECTION OF NATIONAL TUBERCULOSIS ASSOCIATION

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THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

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Membership: All physicians and surgeons in the United States, Canada, and other countries who are interested in pulmonary and other thoracic diseases may apply for membership in the American Trudeau Society. The dues for active membership in the Society are $15* per year, which includes membership in the National Tuberculosis Association, copies of the Society's News Letter, and a subscription to the REVIEW and to the monthly BULLETIN of the National Tuberculosis Association.

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Character of the Review: The REVIEW invites the submission of original manuscripts on any phase of tuberculosis, nontuberculous pulmonary diseases, and related subjects of interest.

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Contributors are requested to pay particular attention to the following rules governing the preparation of manuscripts. Failure to follow these rules is a frequent cause of delay in the publication of articles.

Manuscripts should be sent in duplicate to the office of the Editor, Dr. Walsh McDermott, 1790 Broadway, New York 19, N. Y., mailed flat, and transmitted by first-class mail with postage for return if not acceptable. They should be in English, double-spaced, typewritten on one side of the page with wide margins. Tabular data should not be included in the text but should be presented in separate tables with appropriate reference in the text. All cases of pulmonary tuberculosis should be classified according to the 1955 edition of *Diagnostic Standards and Classification of Tuberculosis* published by the National Tuberculosis Association. All case reports should be written in complete sentences. Authors should exercise particular care in the preparation, notation, and description of figures and tables. The form of reference used in the REVIEW should be carefully followed and references should be checked for accuracy. Each table must be accompanied by a descriptive title, and a suitable legend must be provided for each figure. Drawings must be made with india ink and all lettering should be made with a stencil. The quality of the reproduction of a roentgenogram depends upon both the quality of the original picture and of the glossy print obtained from photographing that picture. If a contributor does not have facilities available, preparation of figures or reproduction of roentgenograms may be handled through the REVIEW office. In those circumstances, the contributor should supply a neat, properly proportioned, and suitably entitled drawing or the original roentgenogram, and will be expected to defray the cost of these services.

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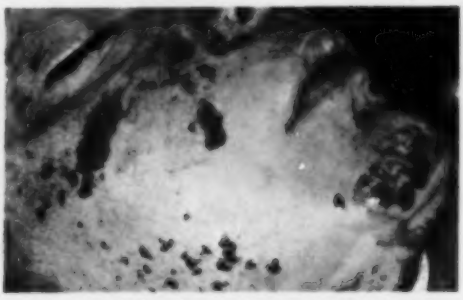

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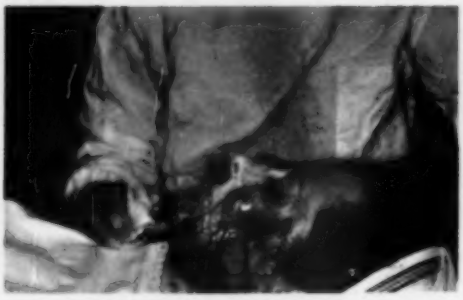

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2. *Ersner, M.S., and Lerner, S.S.: M. Clin. North America 40:1749 (Nov., 1956)*
3. *Peele, J.C.: Further Observations on the Use of Adrenosem Salicylate in the Control of Hemorrhage from the Nose and Throat, N. Carolina M.J. 17:98 (March, 1956)*
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5. *Wilkins, B.D.: Gastrointestinal Bleeding as Seen by the Proctologist, J.A.M.A. 163:1214 (April 6, 1957)*
6. *Orzac, E.: Medical Care of the Child Patient Before and After Adenoidectomy and Tonsillectomy, N.Y. State J. Med. 55:886 (Mar., 1956)*
7. *N.N.R., 1957, p. 265*

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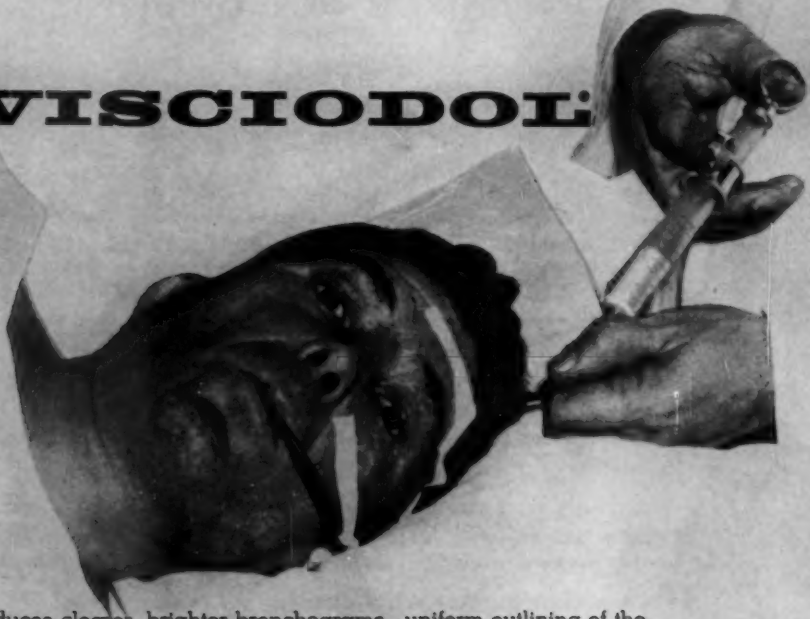

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PHILADELPHIA, PENNSYLVANIA, MAY 18–23

The 1958 Annual Meetings of the National Tuberculosis Association, the American Trudeau Society, and the National Conference of Tuberculosis Workers will be held at Convention Hall, Philadelphia, Pa., May 18 to 23.

The ATS committee meetings will be held Friday, Saturday, and Sunday, May 16, 17, and 18.

Registration will begin Saturday afternoon, May 17, and continue through Sunday evening at the Bellevue-Stratford Hotel. Beginning Monday, May 19, registration, the exhibits program, and regular daytime sessions will be held at Convention Hall. Evening sessions through the week will be held at the Bellevue-Stratford.

Medical, public health, and nursing sessions will begin on Monday, May 19. General sessions will include an opening keynote program Monday A.M., the awards session Wednesday evening, and a session Thursday morning, May 22.

Blocks of rooms have been reserved in the following hotels.

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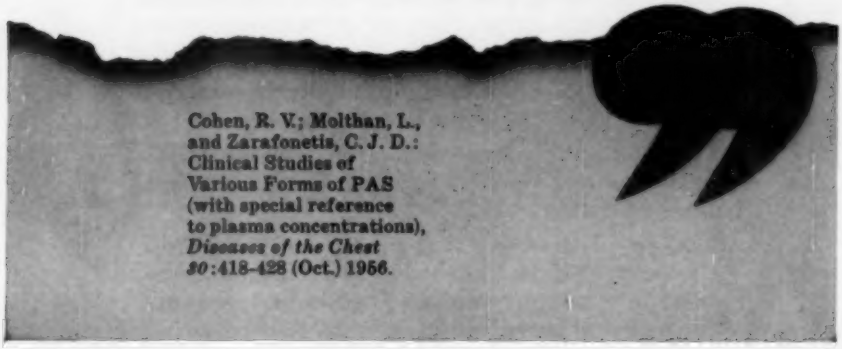

Cohen, R. V.; Molthan, L., and Zarafonetis, C. J. D.: Clinical Studies of Various Forms of PAS (with special reference to plasma concentrations), *Diseases of the Chest* *30*:418-428 (Oct.) 1956.

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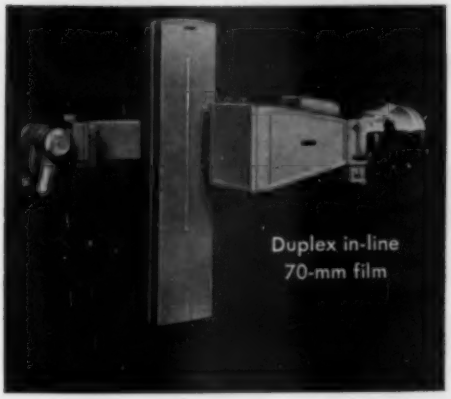

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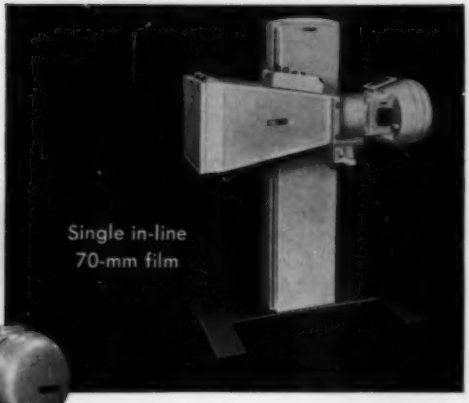

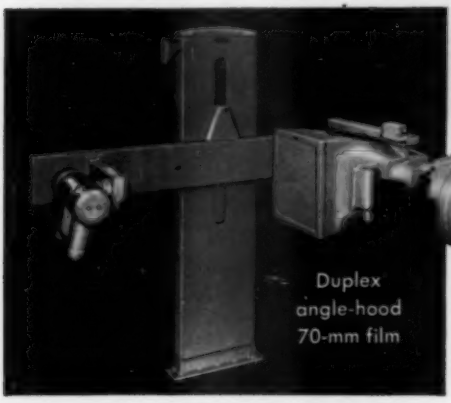

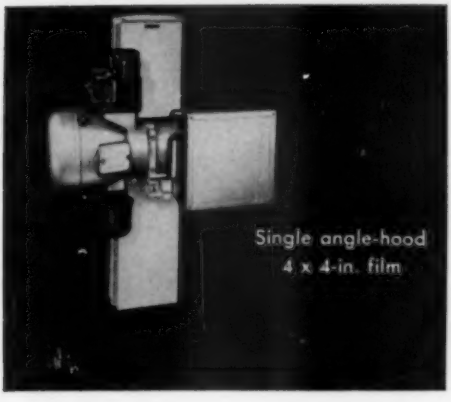

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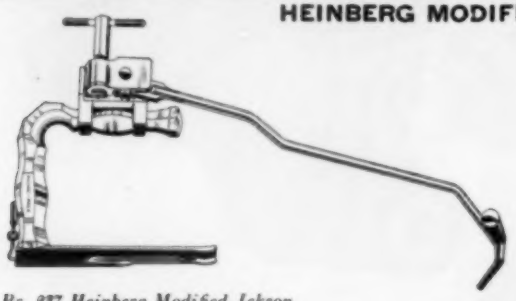

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ERRATUM

In the November, 1957, issue of the REVIEW (volume 76, page 888), the last name of the second author of the Note entitled "The Development of Leukocytic Sensitivity to Tuberculin in Guinea Pigs Experimentally Infected with *Mycobacterium Tuberculosis* H37Rv" was misspelled on the cover and at the end of the Note. It should have been *Scherago* instead of *Sherago*.

On the seventh line of the first paragraph and the fourth line of the fourth paragraph on page 888, footnote *2* should have read footnote *3*.

Letter from the Editor

During the past year the Editor and members of the Editorial Board have called upon many of their colleagues and others especially qualified to review certain manuscripts submitted to the REVIEW for publication. They are most grateful for the help given them by those whose names are listed below:

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THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

VOLUME 77 *January, 1958* NUMBER 1

EFFECTS OF SMOKING UPON THE MECHANICS OF BREATHING[1,2,3]

I. In Normal Subjects

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(Received for publication March 5, 1957)

Introduction

The effects of tobacco smoking in patients with bronchopulmonary disease has been a controversial subject for some time. In a large series of patients, a clinical relationship between smoking and/or chronic cough and bronchitis has been described (1–6). Although the incidence of bronchitis seems to increase with the amount of cigarette smoking, it should be pointed out that, in a series of 310 smokers, 26 per cent of the heavy smokers gave no history of bronchitis. Comparatively few data on actual changes in pulmonary function before and after smoking are available in the literature. Whitfield and associates (7) found, in their studies on lung volumes, that smoking produced a slight diminution in vital capacity and an increase in residual volume and the residual volume/total lung capacity (RV/TLC) ratio. However, they stated that many of the changes observed were due to ageing. Eich and co-workers (8) found no change in the mechanics of breathing in a small group of normal subjects after smoking.

More recently, Loomis (9) reported his belief that cigarette smoke contained a bronchoconstrictor agent demonstrable in guinea pig studies. It is well known, however, that guinea pigs are particularly susceptible to bronchoconstriction. Therefore, these results cannot readily be transferred to humans.

The present study deals with the effects of smoking upon the mechanics of breathing in a series of normal subjects.

Methods and Material

Twenty normal subjects were studied postprandially in the supine position before and after smoking 1 to 2 cigarettes. Measurements were taken during normal respiration and voluntary hyperventilation. The physical characteristics and smoking habits of the group are given in table 1.

[1] From the Department of Medicine, Tufts University School of Medicine, and Lung Station (Tufts); and Department of Inhalation Therapy, Boston City Hospital, Boston, Massachusetts.

[2] This study was made possible by a Grant-in-Aid from the Tobacco Industry Research Committee, New York, New York.

[3] Presented before the Medical Session, as part of *Section 3B*, at the annual meeting of the National Tuberculosis Association, New York, New York, May 22, 1956.

TABLE 1

PHYSICAL CHARACTERISTICS AND SMOKING HABITS IN 20 NORMAL SUBJECTS

	Sex	Age	Body Surface	Vital Capacity	Maximal Breathing Capacity	Smoking Habits
			sq. mm.	*liters*	*L/min.*	*number of cigarettes*
Nonsmokers						
D.S.	M	23	2.38	6.00	—	0
C.W.	M	28	1.87	5.10	145	0
R.M.	M	29	1.76	4.45	106	0
H.P.	M	32	1.90	4.20	151	Stopped smoking for 2 months
G.M.	M	23	2.18	6.10	149	0
J.M.	M	27	2.20	6.20	205	0
R.W.	M	31	1.91	5.20	160	Occasionally 1 cigarette
B.C.	M	24	1.95	5.40	162	0
W.B.	M	33	1.98	5.00	170	0
Smokers						
L.M.	M	27	1.83	5.20	144	10 pipes+
R.B.	M	55	1.77	3.70	84	20+
M.G.	M	30	1.80	4.70	184	20+
C.R.	M	28	1.91	4.10	138	20+
B.C.	M	28	1.80	4.00	122	20+
T.E.	M	32	2.16	5.20	151	20+
J.H.	M	27	2.02	5.20	175	10 pipes+
B.G.	M	25	1.91	5.00	134	20+
A.S.	M	29	1.77	5.00	132	10+
M.W.	M	32	2.14	4.30	97	20+
F.C.	M	27	1.86	5.10	124	20+

The intraesophageal balloon method was employed to measure intrapleural pressure differences as previously described (10). Intraesophageal pressures, air-flow rates, and electrically integrated volumes were continuously recorded. Because of the influence of heart beats, two series of ten cycles each were analyzed, and the results were accepted only if the means of the two series checked. The elastic properties of the lungs are expressed as compliance. This is an index of the degree of lung stiffness and is the inverse of elastance. Compliance is expressed as volume change per cm. of water pressure (L/cm. H_2O). A decrease in compliance represents a less stretchable or more rigid lung and vice versa. The resistive properties of the lungs are expressed as mean mechanical resistance for both inspiration and expiration. The mechanical resistance comprises both the resistance to air flow and the resistance to tissue deformation, and is expressed as cm. of water pressure per liter air flow per second (cm. H_2O/L/sec.).[4] The mechanical work of breathing ($P \times V$) is necessary to overcome the resistances during breathing, the largest component of which is the elastic resistance of the lung.

It is generally assumed that intraesophageal pressure reflects intrapleural pressure quite accurately during normal breathing and hyperventilation in the

[4] Mean mechanical resistance was calculated by dividing resistive pressures by instantaneous air-flow rates at time intervals of one-tenth or one-twenty-fifth of a second. The means were established by graphical integration of the individual values both for the inspiratory and expiratory phases of the respiratory cycle.

sitting position (11–14). There is less agreement (13, 15) about the validity of esophageal measurements in the supine position and after the application of drugs. If the physical characteristics of the esophagus or the mediastinum are suddenly changed, the absolute pressure differences between the intrapleural space and the esophageal lumen must change also. If in addition these physical characteristics vary rhythmically (spasm, et cetera), then not only the absolute pressure difference between the two measuring sites but also the respiratory pressure variations become different.

Therefore, it seemed imperative to verify the validity of the esophageal pressure measurements in the supine position under different experimental conditions. These experiments were carried out in 14 dogs and 6 patients and will be reported in detail elsewhere (14).

Observations

In general, there was good agreement as far as total pressure changes and elastic pressure changes were concerned between intrapleural and intraesophageal measurements during normal respiration and the application of drugs or smoking. Figure 1 is an example of the persistent agreement in intrathoracic pressures in a dog after the injection of nicotine.

The results in 3 subjects immediately after smoking and ten minutes later are shown in figure 2. It will be seen that the values for the two normal subjects were fairly constant and that the changes observed in the patient with chronic bronchial asthma, therefore, were probably not due to changes in mechanics of breathing associated with prolongation of the supine position.

These results were considered good evidence in favor of the assumption that the esophageal method is valid for the evaluation in the changes of mechanics in breathing after smoking.

Results

The results are presented in table 2 for the nonsmokers and table 3 for the smokers. For comparison, mean control values of a group of 25 normal subjects studied in the supine position in this laboratory are given:

Compliance during normal breathing—	0.162 ± 0.040 L/cm. H_2O
Compliance during voluntary hyperventilation	0.150 ± 0.047 L/cm. H_2O
Mean mechanical inspiratory resistance during quiet breathing—	2.95 ± 1.34 cm. H_2O/L/sec.
Mean mechanical expiratory resistance during quiet breathing—	3.45 ± 1.51 cm. H_2O/L/sec.

Analysis of the results in tables 2 and 3 reveals that the changes in the four variables presented in the tables are not significant. Neither is the response of the nonsmoking group as compared to the response of the smoking group (*see* Discussion).

Finally, there are no statistically significant differences between smokers and nonsmokers in the control state.

Comparisons of Intrathoracic Pressures Under the Influence of Drugs

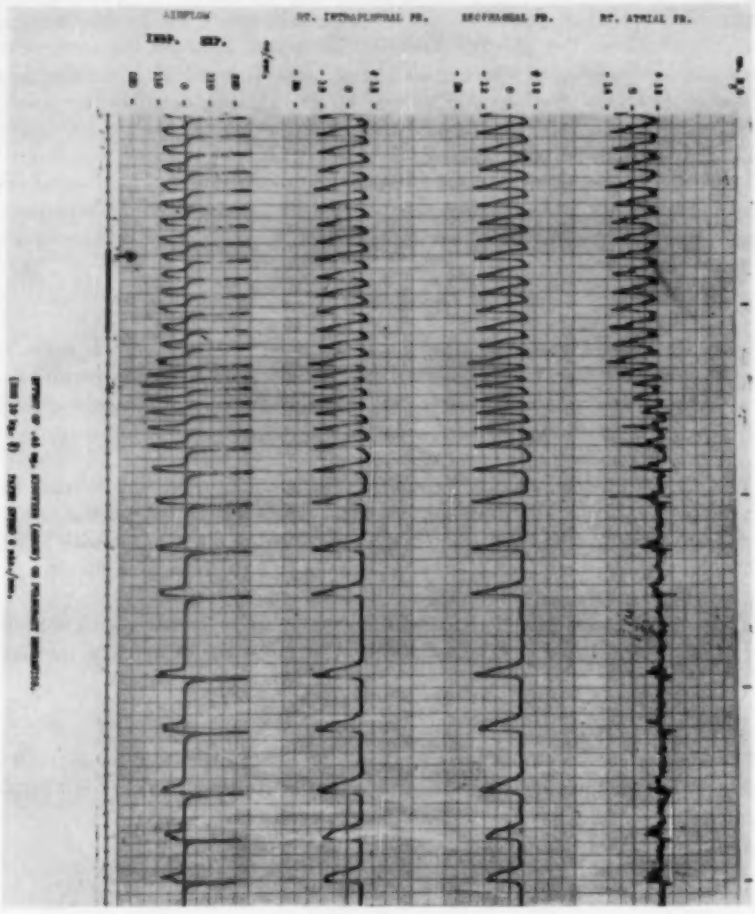

FIG. 1 (Left). Nicotine produces an increase in right atrial pressure with a decrease of pressure variation. Intrapleural and intraesophageal tracings are equal and show slowing of respiration.

DISCUSSION

The results of these studies bring out two interesting facts:

1. The acute effects of smoking upon the mechanics of breathing are in general insignificant for normal smokers and nonsmokers. One nonsmoker (*B.C.*) and two smokers (*C.R.* and *A.S.*), however, showed marked increases in inspiratory mechanical resistance, while two smokers (*B.C.* and *T.E.*) showed

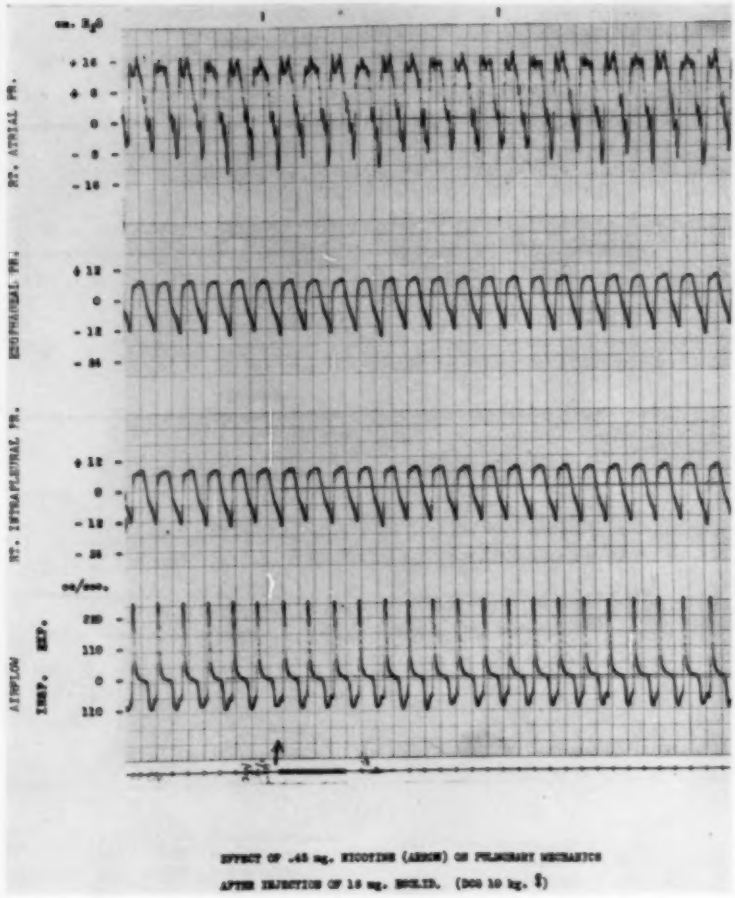

FIG. 1 (Right). Nicotine after Ecolid®. The effect is blocked.

large increases in expiratory resistance. Only one nonsmoker (*B.C.*) and one smoker (*F.C.*) showed decreases in compliance which can be considered significant.

Compliance is independent of respiratory rate up to a cycling frequency of approximately 120 in *normal* subjects. Two nonsmokers (*G.M.* and *R.W.*) and two smokers (*B.C.* and *M.W.*) showed a drop in compliance of 30 to 40 per cent during voluntary hyperventilation after smoking. These individual observations seem to indicate that in some instances smoking may have acute irritative effects upon the pulmonary mechanics in normal subjects, inducing some degree of unequal ventilation.

Comparing the present results with the normal values given in table 2, only

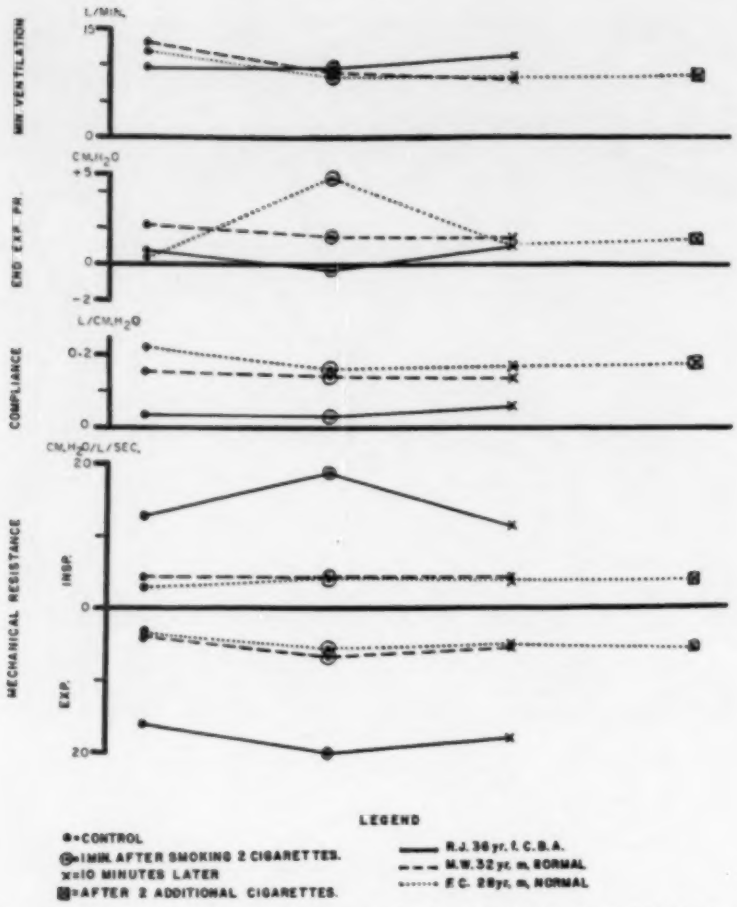

FIG. 2. Effects of cigarette smoking on mechanics of breathing in 3 subjects (supine position).

the decrease in compliance during voluntary hyperventilation (4 subjects) and the highly increased inspiratory resistance of *C.R.* after smoking can be considered abnormal. As has been pointed out in the literature, the chronic cough (attributed to smoking) in smokers disappears after cessation of smoking (1, 3, 5). Unfortunately, the smokers in the present studies, in which the described changes occurred, could not be persuaded to stop smoking for several weeks. Hence, it was not possible to repeat these studies after a period of abstinence.

2. The second fact brought out by these results seems to be quite significant in view of the statements which have appeared in the literature in regard to the

TABLE 2

NORMAL SUBJECTS

Nonsmokers	End Expiratory Pressure		Compliance		Inspiratory Resistance		Expiratory Resistance	
	a	p	a	p	a	p	a	p
D.S.	+0.5	+2.2	0.130	0.103	2.85	3.55	4.35	4.20
C.W.	−2.0	−1.5	0.152	0.193	1.9	2.40	2.90	2.75
R.M.	+1.0	+1.0	0.110	0.106	2.7	3.55	4.45	4.90
H.P.	+2.5	+3.3	0.145	0.130	4.4	4.00	4.60	4.00
G.M.	+2.1	−0.2	0.142	0.175	2.1	2.50	2.40	2.60
J.M.	+1.2	+1.2	0.148	0.124	1.7	2.80	2.50	4.00
R.W.	+0.4	−0.8	0.242	0.255	1.2	1.55	1.80	2.30
B.C.	+2.7	+1.3	0.187	0.131	2.2	5.75	2.20	2.80
W.B.	+7.2	+5.9	0.153	0.125	2.0	5.30	3.45	5.40

TABLE 3

NORMAL SUBJECTS

Smokers	End Expiratory Pressure		Compliance		Inspiratory Resistance		Expiratory Resistance	
	a	p	a	p	a	p	a	p
L.M.	−5.0	−1.0	0.195	0.260	3.00	1.8	2.30	2.80
R.B.	−1.0	−1.0	0.217	0.213	1.00	1.0	2.10	1.40
M.G.	+1.9	+1.9	0.155	0.146	2.80	2.6	2.80	3.90
C.R.	+0.5	+1.5	0.158	0.127	3.95	8.0	6.25	5.00
B.C.	0	+1.5	0.228	0.264	3.45	4.4	3.20	6.60
T.E.	+1.5	+2.0	0.218	0.188	2.10	2.6	2.50	4.80
J.H.	−0.2	0	0.188	0.219	2.65	3.1	2.90	3.10
B.G.	+1.4	+0.8	0.139	0.146	2.95	4.4	3.30	2.30
A.S.	+1.6	+1.7	0.126	0.114	2.20	4.2	1.65	2.20
M.W.	+2.1	+1.5	0.157	0.141	4.30	4.3	3.80	6.60
F.C.	+0.4	+4.8	0.224	0.161	2.95	4.3	3.50	5.75

chronic irritation of the respiratory system by smoking. Statistically, there is no difference in the mechanics of breathing in the control state between smokers and nonsmokers.

This could be due in part to the youth of the subjects (mean, twenty-eight years for nonsmokers and thirty-one for smokers) and the relatively small sample. However, all subjects of the smoker group were heavy smokers (*see* table 1) and, according to the data in the literature, should have developed some respiratory symptoms or defects measurable by these physiologic methods.

SUMMARY

In experiments in humans and dogs, the esophageal balloon method was found to be a valid replacement for direct intrapleural pressure measurements under the influence of drugs and stress conditions. Hence, it was employed for the determination of pressure-flow and pressure-volume relationships in the present studies.

The mechanics of breathing in 20 normal subjects (9 nonsmokers and 11 smokers) were studied before and after smoking one or two cigarettes. There were no statistically significant differences in the mechanics of breathing between smokers and nonsmokers in the control state. Although some subjects showed changes in the mechanics of breathing, statistically indicative of the appearance of unequal ventilation, there was no difference between the control state and the post-smoking state.

Sumario

Efectos del Fumar sobre la Mecánica de la Respiración. I. En los Sujetos Normales

En experimentos en seres humanos y perros, la técnica del globo esofágico resultó ser un substituto válido de las mediciones directas por los sujetos de la presión intrapleural bajo el influjo de las drogas o en condiciones de tensión. Por eso, se empleó en los estudios actuales para la determinación de las relaciones del flujo-presión y del volumen-presion.

La mecánica de la respiración fué estudiada en 20 sujetos normales (9 que no fumaban y 11 que fumaban) después de fumar uno o dos cigarrillos. No hubo diferencias de importancia estadística en la mecánica de la respiración entre fumadores y no fumadores en el período de observación. Aunque algunos sujetos revelaron alteraciones de la mecánica respiratoria, indicativas estadísticamente de la aparición de desigualdad de la ventilación, no hubo diferencia entre el período de control y el período consecutivo al fumado.

Resume

Les effets de la fumée du tabac sur la mecanique respiratoire. I. Chez le sujet normal

Dans l'expérimentation chez l'homme et le chien, la méthode du ballon oesophagien s'est révélée apte à remplacer les déterminations directes de la pression intrapleurale chez les sujets sous l'influence de médicaments ou d'état de stress. Cette méthode a été employée pour la détermination relations débit-pression et volume-pression dans l'étude en cours

Le mecanisme respiratoire chez 20 sujets normaux (9 non fumeurs et 11 fumeurs) fut étudié avant et après que le sujet ait fumé 1 ou 2 cigarettes. Aucune différence significative du point de vue statistique n'a été observée dans le mécanisme respiratoire entre les fumeurs et les nonfumeurs au stade-témoin. Bien que certains sujets aient présenté des modifications dans la mecanique respiratoire indiquant l'apparition d'une ventilation irrégulière, il n'a été noté du point de vue statistique, aucune différence entre le stade-témoin et le stade ultérieur, c'est-à-dire après que le sujet ait fumé.

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EFFECTS OF SMOKING UPON THE MECHANICS OF BREATHING[1,2,3]

II. In Patients with Cardiopulmonary Disease

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(Received for publication March 5, 1957)

INTRODUCTION

In a previous report the effect of cigarette smoking upon the mechanics of breathing in normal subjects was described (1). Significant changes in either compliance or mechanical resistance were not observed in this series. In view of the association claimed between smoking and chronic pulmonary disease (2–10), it was thought that pathologic lungs might respond differently to the inhalation of tobacco smoke. The study was therefore extended to include 25 patients with pulmonary disease and 9 patients with heart disease. In the pulmonary disease group there were 7 patients with chronic bronchial asthma, 8 patients with chronic pulmonary emphysema, 5 patients with chronic pulmonary fibrosis, and 5 patients with miscellaneous pulmonary diseases (3 with bronchitis, one with pleural effusion, and one with a seropneumothorax). The cardiac patients all had rheumatic heart disease with various types of valvular involvement.

The method employed was the same as previously described (1). Continuous measurements of intraesophageal pressure, air-flow rates, and tidal volume were recorded. Pulmonary compliance and mean mechanical resistance over the inspiratory and the expiratory period were determined before and after smoking 1 to 2 cigarettes as described previously (1).

OBSERVATIONS

The results are presented in tables 1 through 5 and in figures 1 and 2. Statistical analysis of this data revealed the following:

In the control state: There was a significantly lower compliance and higher mechanical resistance (expiratory and inspiratory) in the pulmonary and cardiac group as compared with the normal group reported in the previous studies in normal subjects (1). The only significant differences between the pulmonary and the cardiac groups were a less negative (more positive) end-expiratory pressure[4] and a lower compliance in the cardiac group. The former may be indicative

[1] From the Department of Medicine, Tufts University School of Medicine, and Lung Station (Tufts); and Department of Inhalation Therapy, Boston City Hospital, Boston, Massachusetts.

[2] This study was made possible by a Grant-in-Aid from the Tobacco Industry Research Committee, New York, New York.

[3] Presented before the Medical Session, as part of *Section 3B*, at the annual meeting of the National Tuberculosis Association, New York, New York, May 22, 1956.

[4] In the supine position, end-expiratory, esophageal pressures are less negative than in the sitting position. Therefore, they can not be compared directly with values obtained in other body positions.

TABLE 1

CHRONIC PULMONARY EMPHYSEMA

	End Expiratory Pressure		Compliance		Inspiratory Resistance		Expiratory Resistance	
	a	þ	a	þ	a	þ	a	þ
Nonsmokers								
M.J.	+0.25	+0.3	0.118	0.150	4.2	3.55	4.7	3.40
R.D.	+3.80	+2.0	0.100	0.066	7.8	11.40	14.9	16.60
Q.D.	+2.60	+4.4	0.079	0.078	15.3	14.90	25.9	33.00
H.H.	−0.50	−0.8	0.250	0.191	5.7	6.85	8.5	17.75
Smokers								
B.G.	0.0	+5.5	0.047	0.050	14.3	14.5	23.5	27.50
F.F.	−2.5	−3.0	0.089	0.083	11.1	7.4	24.3	26.60
M.N.	+0.2	−0.5	0.110	0.080	6.5	9.4	9.4	12.75
J.B.	−2.2	−1.8	0.281	0.245	7.4	8.4	17.1	19.00

TABLE 2

CHRONIC BRONCHIAL ASTHMA

	End Expiratory Pressure		Compliance		Inspiratory Resistance		Expiratory Resistance	
	a	þ	a	þ	a	þ	a	þ
Nonsmokers								
M.F.	0.0	+0.5	0.190	0.160	3.80	4.60	8.4	8.2
H.P.	−2.4	0.0	0.100	0.119	4.45	4.50	5.3	4.8
E.S.	0.0	+0.5	0.087	0.114	5.95	5.60	13.7	14.2
H.S.	+1.0	0.0	0.136	0.148	3.00	3.05	4.6	4.1
Smokers								
E.W.	+1.5	−3.7	0.100	0.100	7.5	6.1	7.4	9.95
R.J.	+0.5	−0.5	0.038	0.034	12.8	18.2	16.0	19.90
H.P.	−1.8	+1.4	0.098	0.113	24.0	24.6	33.8	56.80

of a lower mid-position. In addition, the patients with chronic bronchial asthma, chronic pulmonary emphysema, and bronchitis showed a characteristic decrease of pulmonary compliance during voluntary hyperventilation.

Response to smoking in various groups: The only significant differences found were a rise in expiratory resistance in the emphysema group ($P < 0.02 < 0.01$) coupled with a small decrease in compliance. In all of the other groups, none of the variables studied showed a significant change. In particular, no variations in the decrease of compliance during voluntary hyperventilation were observed. If the individual results are considered, there was a considerable increase in respiratory mechanical resistance in 3 patients (*R.J.*, *A.deW.*, and *G.A.*). The first of these had chronic bronchial asthma, the second pulmonary fibrosis, and the third pleural effusion. Expiratory resistance rose markedly in 2 patients with chronic pulmonary emphysema (*Q.D.* and *H.H.*), in one patient with chronic bronchial asthma (*H.P.*), and in one patient with mitral stenosis associated with chronic pulmonary emphysema (*T.E.*).

TABLE 3

PULMONARY FIBROSIS

	End Expiratory Pressure		Compliance		Inspiratory Resistance		Expiratory Resistance	
	a	p	a	p	a	p	a	p
Nonsmokers								
C.F.	−1.5	+0.5	0.077	0.070	1.10	1.05	1.60	2.90
C.F.	+0.5	+0.5	0.113	0.118	1.85	1.44	4.75	2.65
Smokers								
A.deW.	+1.5	+3.0	0.045	0.048	12.45	20.40	19.35	19.05
T.E.	−2.0	+1.0	0.150	0.125	3.40	4.85	5.15	6.65
J.D.	+2.5	−2.0	0.039	0.056	4.40	3.60	11.70	7.00

TABLE 4

MISCELLANEOUS PULMONARY DISEASES

	End Expiratory Pressure		Compliance		Inspiratory Resistance		Expiratory Resistance	
	a	p	a	p	a	p	a	p
Smokers								
H.P.	+1.0	+1.0	0.169	0.151	2.6	4.80	5.3	4.70
G.A.	+1.5	+4.5	0.105	0.082	5.7	10.90	10.6	18.10
J.M.	−2.0	−2.2	0.162	0.123	6.2	7.40	16.5	16.60
B.B.	+3.2	+1.9	0.097	0.107	3.2	3.80	4.6	4.80
S.H.	+4.2	+4.4	0.044	0.046	3.1	2.75	4.8	5.35

TABLE 5

HEART DISEASE

	End Expiratory Pressure		Compliance		Inspiratory Resistance		Expiratory Resistance	
	a	p	a	p	a	p	a	p
Nonsmokers								
S.P.	+2.00	+2.00	0.068	0.074	7.6	10.80	12.6	12.6
A.C.	+2.25	+4.75	0.066	0.060	5.3	6.60	8.7	8.1
O.L.	+1.20	+1.25	0.058	0.064	2.1	2.15	5.3	5.7
D.S.	+5.60	+6.80	0.053	0.046	4.9	6.50	4.7	8.7
C.B.	+14.70	+3.10	0.025	0.039	13.5	8.70	21.3	21.0
Smokers								
T.D.	−2.2	−1.75	0.113	0.111	6.1	8.90	9.3	9.75
T.F.	−0.5	0.00	0.136	0.102	4.3	6.55	7.8	16.80
A.C.	+2.1	−2.00	0.092	0.064	3.1	2.90	2.8	3.80
C.B.	+7.7	+1.25	0.045	0.057	4.5	3.90	14.8	10.60

As a corollary, there was no significant difference in the response to smoking between the various groups, except in the patients with chronic pulmonary emphysema.

Difference between smokers and nonsmokers: In the pulmonary group, the

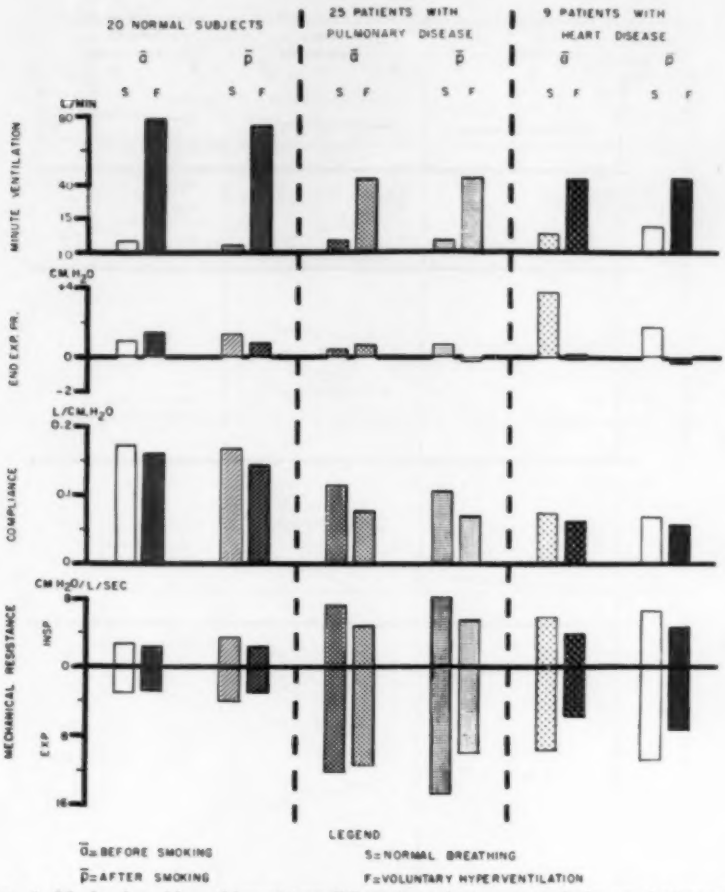

FIG. 1. Mechanics of breathing at two different respiratory rates before and after smoking in three different groups of patients (mean values, supine position).

relative effects of smoking in established smokers and nonsmokers could not be meaningfully evaluated within each separate disease entity because of inadequate sample size. Therefore, all the pulmonary patients were included as a single group and the effects of smoking between the smokers and nonsmokers were compared. There were no significant differences between the smokers and nonsmokers in the normal and in the cardiac groups. On the other hand, in the pulmonary group, expiratory resistance was somewhat higher in the smoker as compared to that in the nonsmoker (P approximately = 0.1). It will be seen in figure 3 that this increased resistance is present in each pulmonary group. On the basis of these results, it is impossible to tell whether these differences are really related to smoking habits or to the selection of patients.

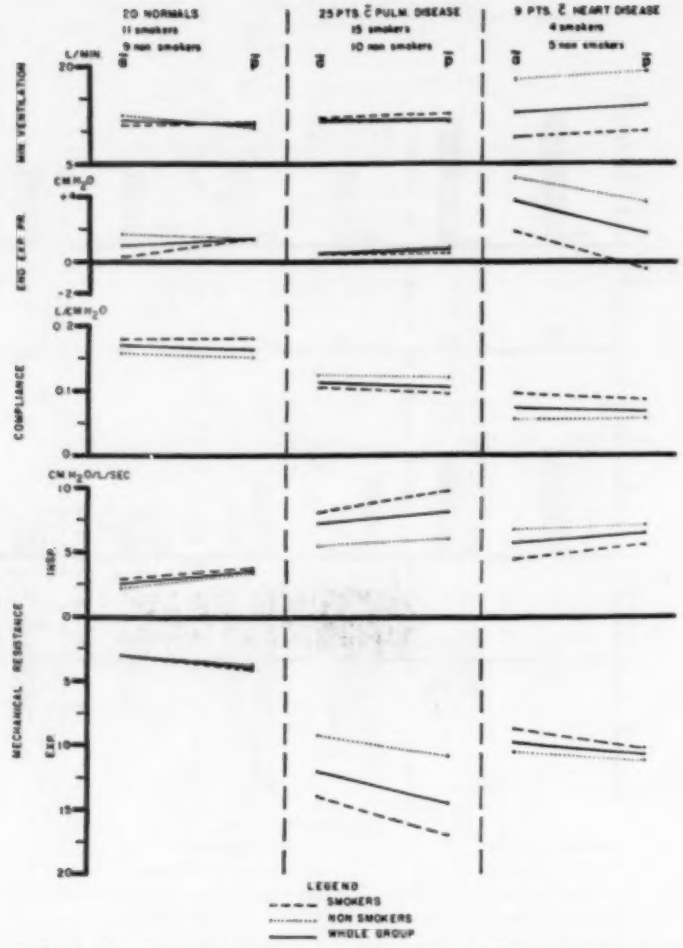

FIG. 2. Mechanics of breathing before (ā) and after (p̄) smoking in different groups of subjects, according to smoking habits. (Mean values, supine position).

DISCUSSION

The present findings are somewhat surprising in the light of the clinical findings in the literature. In essence, they confirm the results of Eich and associates (11). These investigators, using essentially the same technique, found a marked increase in mechanical resistance in patients with chronic pulmonary emphysema, but no changes in normal subjects.

Any difference noted between smokers and nonsmokers in the control state would indicate the chronic effects of smoking upon the mechanics of breathing, provided the groups are otherwise comparable.

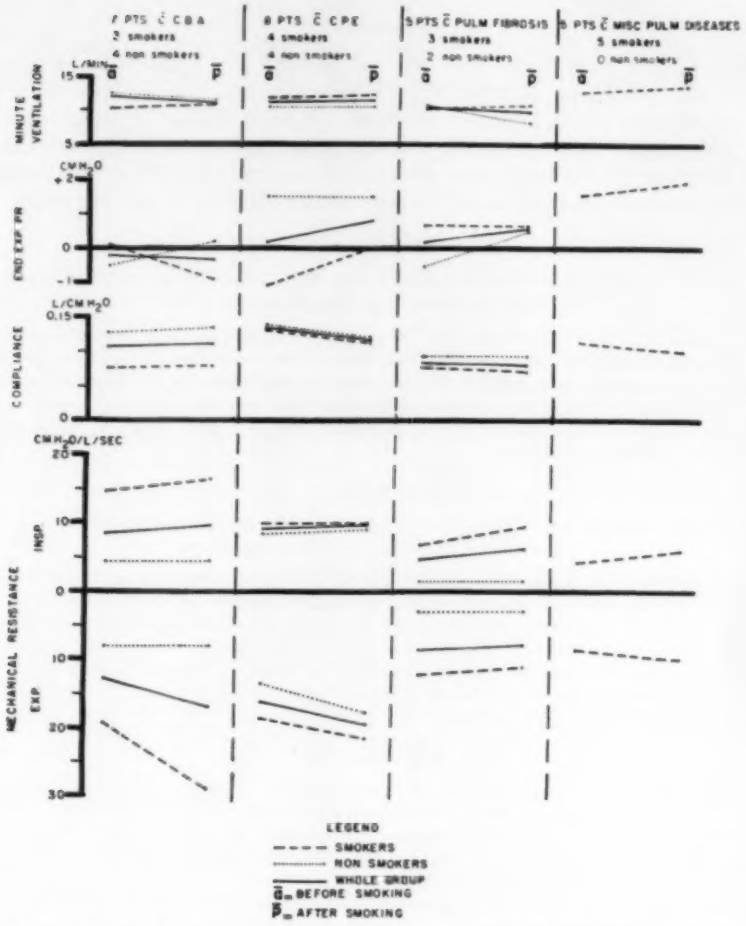

FIG. 3. Mechanics of breathing before and after smoking in different groups of pulmonary patients according to smoking habits. (Mean values, supine position).

The total number of persons studied in each of the three main groups studied permits valid statistical analysis. The over-all results in the normal subjects and in patients with cardiac disease fail to show any *marked difference* between smokers and nonsmokers. An increase in mechanical resistance was noted in the smokers in the pulmonary disease group. The harmful physiologic effects of smoking noted in some patients therefore seem to be dependent upon the individual. These effects may be accentuated in patients with chronic involvement of the tracheobronchial tree, such as those seen in chronic pulmonary emphysema.

Summary

Smoking one or two cigarettes did not produce any significant changes in mechanical resistance, pulmonary compliance, or the work of breathing in normal subjects and in patients with rheumatic heart disease. However, significant physiologic changes, characterized by an increase in mechanical resistance and the work of breathing, were observed in patients with chronic pulmonary emphysema and other pulmonary diseases associated with chronic bronchitis.

Sumario

Los Efectos del Fumar sobre la Mecánica de la Respiración. II. En Enfermos que Tienen Afecciones Cardiopulmonares

El consumo de uno o dos cigarrillos no produjo la menor alteración de la resistencia mecánica, la participación pulmonar o el trabajo de la respiración en sujetos normales y en enfermos con cardiopatía reumática. Sin embargo, se observaron importantes alteraciones fisiológicas, caracterizadas por aumento de la resistencia mecánica y del trabajo de la respiración, en enfermos que tenían enfisema pulmonar crónico u otras afecciones pulmonares vinculadas con bronquitis crónica.

Resume

Les effets de la fumée du tabac sur la mecanique respiratoire. II. Chez les malades atteints d'affections cardiopulmonaires

Après qu'une ou deux cigarettes aient été fumées il n'y a eu aucune modification significative de la résistance mécanique, et de la participation pulmonaire au travail de la respiration chez le sujet normal ou chez le malade atteint d'une affection cardiaque rhumatismale. Toutefois, des modifications physiologiques significatives, caractérisées par une augmentation de la résistance mécanique et du travail dans la fonction respiratoire furent observées chez les sujets atteints d'emphysème pulmonaire chronique ou d'autres affections du poumon associées à la bronchite chronique.

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THE SURGICAL TREATMENT OF PULMONARY COCCIDIOIDOMYCOSIS WITH A COMPREHENSIVE SUMMARY OF THE COMPLICATIONS FOLLOWING THIS TYPE OF THERAPY[1]

DERMONT W. MELICK[2]

(Received for publication May 28, 1957)

Introduction

This paper is a report on 400 cases of coccidioidomycosis treated by surgery. Sixty-six different surgeons participated, including the present writer.

The data for the most part were gathered by the questionnaire method. One such questionnaire was sent out in 1950 (1) and a second in 1953. A third questionnaire for 1957 has not been completed. Some of the 1957 data are, nevertheless, used herein in order to bring the report up to date. In the 1950 survey (1), ninety-eight cases were reported. Three hundred and two cases have been added since then. Cotton, Paulsen, and Birsner (2) reported the largest series (110 cases). Forsee and Perkins (3) reported 55 cases, and another 54 of the 400 cases were treated by the present writer.

Observations

Indications for Surgical Therapy

The indications for surgical therapy are hereby presented in a somewhat arbitrary grouping, but it is hoped this will aid in clarification.

Absolute Indications—Group I
- *a.* Giant cavity (more than 5 cm.)
- *b.* Infected cavity
- *c.* Ruptured cavity
  - *1.* Bronchopleural fistula
  - *2.* Empyema
  - *3.* Nonexpansile lung
  - *4.* Persistent pneumothorax

Probable Indications—Group II
- *a.* Enlarging cavity
- *b.* Cavity with hemorrhage

Possible Indications—Group III
- *a.* Coin lesion
- *b.* Persistent cavity
- *c.* To establish diagnosis
  - *1.* Tumefaction
  - *2.* Other inflammatory lesions: tuberculosis, histoplasmosis, blastomycosis.

The *absolute indications* under *Group I* will cause very little comment, as the necessity for surgery is obvious.

[1] Presented before the Medical Session, as part of *Section 5*, at the annual meeting of the National Tuberculosis Association, Kansas City, Missouri, May 8, 1957.

[2] Present address: 1005 Professional Building, Phoenix, Arizona.

The *probable indications* under *Group II* are those cases with definite warning signs of impending complications. Definitive surgical therapy is considered to be indicated. An enlarging cavity, particularly if it is near the visceral surface of the lung, may rupture. This not infrequently results in a pneumothorax. The more disturbing sequelae of rupture are bronchopleural fistula, empyema, and nonexpansile lung. Occasionally, a patient may be fortunate enough to have only a minimal pleural effusion following rupture. Unfortunately, this is rare. Intracavitary hemorrhage may be severe. Recurrent hemorrhage in a cavity usually demands surgical extirpation.

The *possible indications* (*Group III*) will not be universally accepted among thoracic surgeons. Most thoracic surgeons are inclined to the well-founded belief that all "coin lesions" demand early thoracotomy in order to establish the diagnosis with accuracy. Emphasis has been placed on the high percentage of malignancy in the coin lesion. This varies from 15 to 35 per cent.

The "coin lesion" due to coccidioidomycosis is a frequent finding in the endemic area. It is estimated that the "coin lesion" in the endemic area will be found to be malignant in *less than 5 per cent* of cases. The statistical possibility that the "coin lesion" is malignant is thereby greatly reduced. The decision to be made by the individual surgeon in such an area is whether this reduction in percentage justifies conservatism.

It would appear, therefore, that the "coin lesion" in the endemic area should be given more scrutiny than the "coin lesion" in other geographic areas. This would imply a careful history of past febrile episodes, adequate skin testing (with particular attention to the 1:100 and 1:10 coccidioidin), plus blood-agglutination and complement-fixation tests. It is believed by some that the roentgenographic appearance exhibits characteristics highly suggestive of coccidioidomycosis. The disease is not infrequently located near one of the pulmonary fissures and it has a propensity for burrowing across the fissure from one lobe to another. This "*crossing the fissure*" sign lends weight to a presumptive diagnosis of coccidioidomycosis. If, after careful search, the diagnosis of the "coin lesions" remains indeterminate, the decision for exploratory thoracotomy will not be questioned. Caution, however, should be exercised by the surgeon when removing the "coin lesion." In one report (5) of resections for "coin lesions," the surgeon performed lobectomy in three of four cases. In order to prevent undue loss of lung tissue, a more localized excision may be advisable to establish the diagnosis.

The second *possible indication* is persistent cavitation. Depending on the size of the cavity, most physicians (and even some surgeons) would prefer to treat such a case conservatively. Certainly if the cavity is newly discovered or small in size, conservatism is justified. The cavity may disappear and surgery be unnecessary. Some California surgeons believe disappearance of a cavity is wishful thinking and that "recavitation" in the same area will occur sooner or later. Winn, however, found cavity closure to occur in 25 per cent of his first ninety-two patients. Cavitary lesions have been observed to remain the same size for months

and even years (6). Cotton, Paulsen, and Birsner have stated that they have never observed spontaneous closure of a cavity that has persisted six months. The present writer, on the contrary, has observed one case of bilateral cavitation with gradual decrease in size of both cavities and eventual disappearance during a period of two and one-half years. Certainly, if a cavity persists for twelve to eighteen months without closure and specifically if it is not definitely decreasing in size, the chances of its closing spontaneously are remote indeed. If a physician happens to have a cavitary lesion, it is revealing to observe how he treats it. Two of the writer's colleagues have treated such cavities conservatively and, to date, they are in good health without benefit of surgical intervention. One should also bear in mind that the complications reported in the latter part of this paper have been found to follow cavitary lesions much more frequently than to follow surgery on solid lesions. This may be explained by the fact that the mycelial form of *Coccidioides immitis* is present in 50 per cent of cavitary cases and in only 10 per cent of solid lesions (5).

Comprehensive Summary of the Complications Following Surgical Therapy

The complications following surgery for coccidioidomycosis are probably higher than might be surmised from reading the reports in the literature. A recent article (4) emphasizes this suspicion. Complications were experienced in three of the seven patients operated upon.

The 400 cases herein reported have had a complication rate of 13 per cent (seven deaths occurred, a mortality rate of 1.7 per cent). The 54 personal cases of the writer included in the above report reveal a complication rate of 18 per cent (no deaths occurred in this group). Cotton, Paulsen, and Birsner have had the best record with only five complications in 110 cases. Forsee and Perkins, in their 55 cases, reported five complications.

The complications range from persistent postoperative pneumothorax through empyema and nonexpansile lung to bronchopleural cutaneous fistula and multiple chest wall sinuses. In the 54 cases about which the present writer has detailed knowledge, the ten complications can be divided into five major and five minor complications. The major complications were as follows:

1. Bronchopleural cutaneous fistula with multiple chest wall sinuses
2. Bronchopleural cutaneous fistula with empyema characterized by debilitation and invalidism of the patient
3. Recurrence of cavity resulting in bronchopleural cutaneous fistula following secondary operation
4. Bronchopleural fistula with empyema requiring open drainage and followed by a prolonged period of healing
5. Persistent cavitation at the same site of removal of the original cavity

The five minor complications included two cases of empyema treated successfully by repeated thoracentesis; one case of persistent minimal hemoptysis (present over a period of fifteen months) thought to be due to a small cavity in the same area as the original resection with eventual spontaneous closure of cavity

and cessation of hemoptysis; progressive pneumonitis in the same area as the resection with evidence of a small cavity with eventual spontaneous resolution and cavity closure; and one case of persistent pneumothorax following resection.

The over-all complication rate of 13 per cent may be expected to continue as long as the etiologic agent is not controlled by any of the known antimicrobial drugs. Despite the complications, there is no doubt that surgery has a definite place in the treatment of pulmonary coccidioidomycosis.

Summary

Surgical therapy in pulmonary coccidioidomycosis is a well-recognized procedure within the indications herein outlined. Four hundred cases have been collected. Especial attention has been given to the rate of complications in this group of cases, which has been found to be 13 per cent. In certain case reports by other writers, the complication rate is less than 5 per cent. In the present report, a group of 54 cases is reviewed, in which the complication rate was 18 per cent. Complications, when they do occur, can be expected to be severe in one of each two cases. These complications, major or minor, are a confirmation of the fact there is no good antifungal agent to assist in the protection of the patient when surgery is performed. The necessity for surgery will be lessened when such an antifungal agent is available.

Sumario

El Tratamiento Quirúrgico de la Coccidioidomicosis Pulmonar con un Minucioso Sumario de las Complicaciones Consecutivas a esta Forma de Terapéutica

La terapéutica quirúrgica de la coccidioidomicosis pulmonar es un procedimiento bien reconocido dentro de las indicaciones aquí bosquejadas. Se ha prestado atención especial al índice de complicaciones en este grupo de casos, resultando ser de 13 por ciento. En ciertas casuísticas de otros autores, el índice de complicaciones ha sido de menos de 5 por ciento. En la comunicación actual, repásase un grupo de 54 casos, en el que el índice de complicaciones es de 18 por ciento. Cabe esperar que las complicaciones, cuando se presentan, sean graves en uno de cada dos casos. Estas complicaciones, mayores o menores, ofrecen confirmación del hecho de que no hay ningún buen fungicida que ayude a proteger al enfermo cuando se utiliza la cirugía. Cuando se cuente con tal fungicida, habrá menos necesidad de la cirugía.

Resume

Traitement chirurgical de la coccidioidomycose et résumé de l'ensemble des complications succédant à ce mode de traitement

Le traitement chirurgical dans la coccidioidomycose est un procédé très classique dans le cadre des indications décrites dans cet article. Quatre cents cas ont été réunis. L'accent est mis sur le pourcentage des complications dans ce groupe; il s'élève à 13%. Dans certains rapports communiqués par d'autres auteurs, le taux des complications est inférieur à 5%. Le rapport actuel concerne 54 cas pour lesquels le taux des complications est de 18%. Lorsque ces complications surviennent, il y a lieu de prévoir qu'elle seront graves dans un cas sur deux. Ces complications, majeures ou mineures, confirment le fait qu'il n'y a pas d'agent fungicide satisfaisant qui assure la protection du patient soumis à l'acte chirurgical. La nécessité de la chirurgie sera moindre quand on disposera d'un tel agent fungicide.

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CYTOLOGIC PATTERNS IN BRONCHOPULMONARY DISEASE[1]

A. ALBERT CARABELLI

(Received for publication July 31, 1957)

Introduction

The present study was conceived with the purpose of determining whether there existed pathognomonic patterns in the cytologic exudates and exfoliates of the various bronchopulmonary diseases which might be characteristic from a diagnostic point of view. Special emphasis was placed on cell types and patterns exclusive of bacteriologic or mycologic findings. It was thought that, since much information had been gathered in the neoplastic diseases of the lung by such study, perhaps similar information could be applied to the diagnosis of other types of bronchopulmonary diseases.

Materials and Methods

The source material for this study consisted of specimens collected endoscopically from 1,000 patients with some manifestation of bronchopulmonary disease. All cases were personally followed, especially the doubtful cases, up to a period of six years. Many "normal" cases were encountered, but these were included for the purpose of comparison and to study normal variants.

Specimens were collected by the use of a special instrument devised by the writer (1) (figure 1)[2] which ensured the obtaining of secretions directly from or close to the disease focus uncontaminated by secretions from the mouth, nasopharynx, trachea, or large bronchi. Since the instrument permitted collection from a 2 mm. straight or curved bronchus, the presence of alien cells was fairly well eliminated. This instrument has a dual function, since it can be used as a direct aspirator or as an irrigator-aspirator when the secretions are scanty or not apparent. An added feature of the instrument is the small corrugated flexible tip which permits a scraping motion, with gentle trauma of the bronchial wall, while the sterile saline is being instilled through the irrigating insert. Superficial cells and sometimes deeper cells are loosened from the wall, floated in the saline, and aspirated into the reservoir for special study.

With this technique, two types of specimens are obtained: one a direct aspirate, and the other an irrigate with a suspension of cellular elements in saline. In the case of the direct aspirate, smears are made for tubercle bacilli and for eosinophils (Wright's stain). Specimens are processed for guinea pig inoculation and cultures for tubercle bacilli. Cover-slip smears are made for the Papanicolaou technique. In the case of the irrigate, the same procedure is followed except that the specimens for the guinea pig inoculation and culture are taken first (one half of the irrigate), and the remainder is centrifuged, and smears for tubercle bacilli, eosinophils, and Papanicolaou studies are made from the decanted sediment.

This procedure represents a more refined technique for the diagnosis of bronchopulmonary disease and the writer has found the technique to be of immense value in the detection of early phases of such disease.

To properly evaluate the types of cells found, a cytogram form was devised (figure 2). Accurate quantitative determination obviously could not be made because both aspirates and irrigates were being used; and dilution factors would render the results grossly in-

[1] From the Department of Thoracic Medicine, St. Francis Hospital, Trenton, New Jersey.

[2] This collector is made by the George P. Pilling & Sons Company, 3451 Walnut Street, Philadelphia, Pennsylvania.

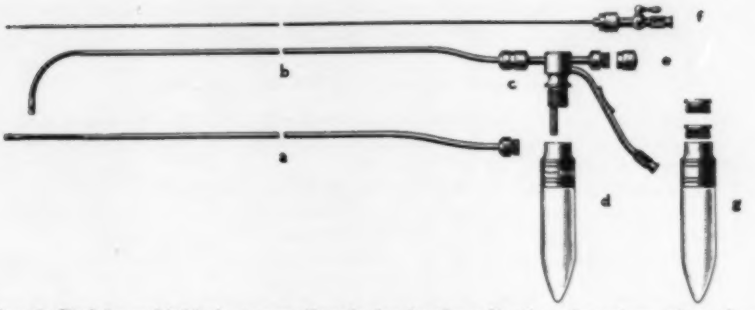

FIG. 1. Endobronchial irrigator-suction device for the collection of specimens from deep in the bronchial tree.

a. Straight, flexible-tip collecting tube
b. Curved, flexible-tip collecting tube
c. Collecting head
d. Reservoir
e. Closure
f. Irrigation insert
g. Extra reservoir and closure

CYTOGRAM
(Papanicolaou Stain)

Name Date

Diagnosis

( )Bronchial aspirate ( )Bronchial irrigate

CLASSIFICATION (Plus)	1	2	3	4
Leukocytes				
Erythrocytes				
Lymphocytes				
Eosinophiles				
Plasma cells				
Ciliated columnar				
Goblet cells				
Undifferentiated basal cells				
Histiocytes (macrophage)				
Squamous cells				
"Papanicolaou" cells				
Pseudomalignant types				
Malignant types				

REMARKS

FIG. 2. Form of cytogram to evaluate the types of cells.

accurate. A rough quantitative classification was made by indicating plus 1, 2, 3, or 4 opposite the type of cell found.

Types of Bronchopulmonary Diseases Studied

The following types of diseases were encountered and are here listed to give some concept of the variety of the work. Both the bronchopulmonary diseases usually seen and some of the rarer ones are listed:

Bronchosinusitis and bronchitis, acute, subacute, and chronic

Acute suppurative bronchopneumonitis, obstructive in type, such as acute abscess, acute suppurative atelectasis secondary to foreign body, or idiopathic

Chronic suppurative bronchopneumonitis, obstructive in type, such as chronic abscess, bronchiectasis, chronic suppurative pneumonitis

Simple atelectasis of the segmental, lobar, or massive types

Allergic diseases such as bronchial asthma and Löffler's syndrome

Congenital anomalies, such as infected cystic disease of the lung; hypogenesis of the right lung with absence of the right upper and middle lobes; and hereditary, familial telangiectasia of the pulmonary system

Pneumoconiosis both of the anthracotic and silicotic types

Neoplastic disease of the lungs and bronchi, either intrinsic or metastatic, such as bronchogenic carcinoma, superior sulcus tumor, lymphoma, lymphoblastoma, Hodgkin's disease, sarcoma, myelogenous and lymphogenous leukemia

Bronchostenosis, either tuberculous, nonspecific, or due to foreign body

Tuberculosis, acute, chronic, or arrested; including cases of artificial pneumothorax, extrapleural pneumothorax, or thoracoplasty

Empyema of tuberculous or other etiology; emphysema of the bullous or fibrous variety

Fungal disease

Observations

The normal cytogram and its normal variations: Included in what is, for practicable purpose, a normal cytogram are the following types of cells: ciliated columnar; goblet cells; undifferentiated bronchial epithelial cells from the lower mucosal layers; histiocytes (macrophages); and squamous cells (figure 3). Blood elements are usually due to instrumental trauma and are not considered in the normal picture.

The predominating cell is the *ciliated columnar* which is characterized by the presence of cilia, a cuticular membrane, and an elongated columnar body which ends in a tapered tail. The cytoplasm proper shows a fine reticular or granular pattern and may contain a few vacuolations. The nucleus is usually ovoid, with a rather delicate chromatin network with chromatin granules in the strands. The nucleolus is not usually prominent and in some cases cannot be seen. Occasionally a small nipple-like protrusion or herniation of the nucleus into the cytoplasm toward the cilia is seen. At other times a clear zone or "halo" separates the nucleus from the cytoplasm proper. Anisocytosis and poikilocytosis are relatively common. The cell may be perfectly round and small with a tuft of cilia projecting fanlike from the periphery; it may be barrel-shaped and squatty or tremendously elongated. Binucleate forms are fairly common, and multinucleated forms are rarer. The normal ciliated forms may be observed singly in great numbers spread throughout the smear, or occasionally they may be grouped

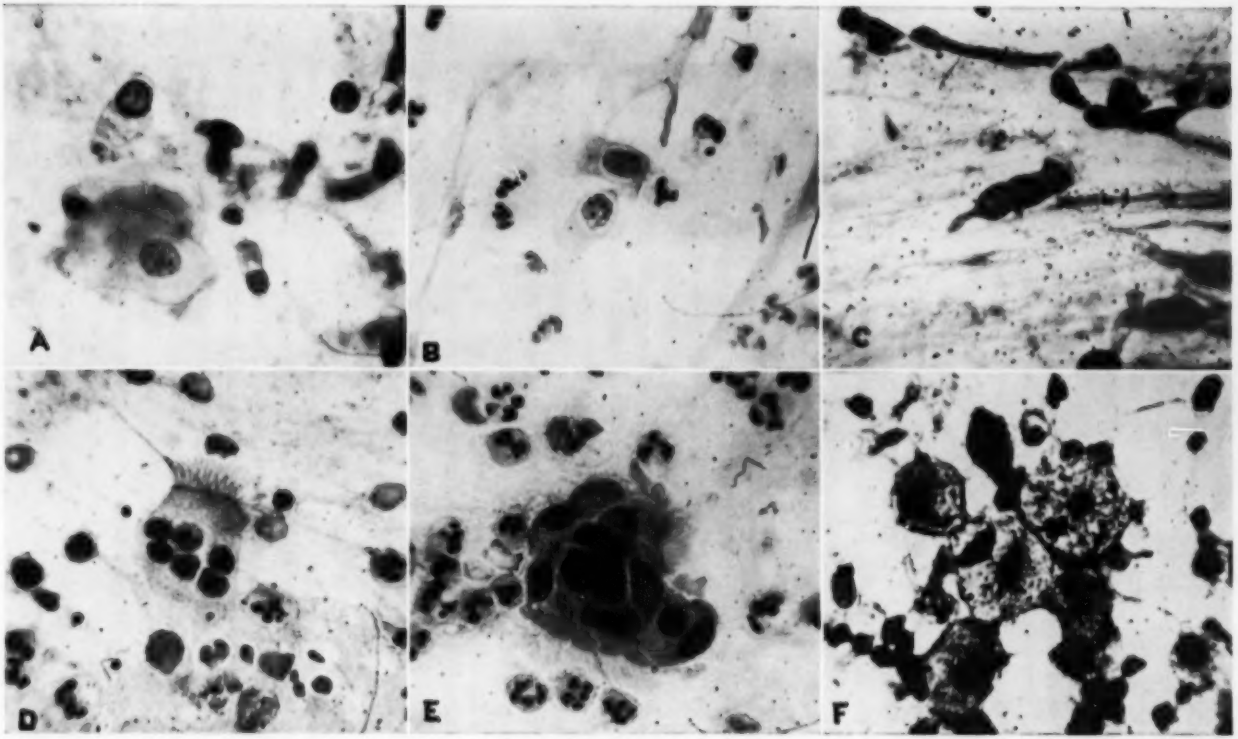

FIG. 3. Cellular elements and their variants seen in the normal cytogram:

A. Typical assortment of normally found cells. Note aspirated squamous cell, ciliated columnar and goblet cells. These latter are bulging with mucoid inclusions.

B. Normal ciliated types with "halo" about the nucleus.

C. Binucleate form of ciliated columnar cell.

D. Multinucleated form of ciliated columnar. Note distinct loss of individual cell demarcations.

E. Ciliated group with anisokaryosis, easily mistaken for the malignant type except for the presence of cilia.

F. Histiocytes loaded with carbon particles.

in a phalanx much as they are in tissue sections. At times groups may be seen which contain the basal cell layers as well as the superficial columnars.

Goblet cells are also of the columnar variety but contain mucous inclusions which usually distend the cell and crowd the nucleus to the base or laterally and at times distort its ovoid outline and flatten one surface. These cells possess no cilia and are easily identified.

The aspirated *squamous cell* is not properly a resident of the tracheobronchial tree in normal specimens, but represents an alien cell which is occasionally aspirated deep into the bronchi. This cell has all the characteristics of the squamous cell in its normal habitat. It is found in types varying from the dry-appearing scale with the crinkled cellophane appearance, with no nucleus or "ghost" nucleus, to a well-defined and healthy cell with a nucleus with visible chromatin network and nucleolus. Occasionally fairly large sheets of these cells are found as a microscopic membranous slough. *Epithelial pearls*, although rare, may be expected to be found in normal specimens.

A very common cell to be found is the *histiocyte* (*macrophage*) which is ubiquitously present as the scavenger of the lung in both normal and disease specimens. No diagnostic import can be attributed to this cell since it may be found in great numbers in normal cases. This cell is rather large, spherical in shape, with a reticulated cytoplasm. Cytoplastic inclusions may consist of vacuolations, pigment particles, or even small cells in varying proportions. In some cases the cell is heavily loaded with carbon when it gives a very distinctive appearance. The nucleus may be central or eccentric, and binucleated forms are common. It may be pyknotic or well defined with prominent nucleoli.

From the clinical point of view most of the diagnostic difficulties center about groups of columnar cells which are arranged erratically and superimposed or are undergoing degenerative changes. These may be confused easily with malignant types because the cilia have disappeared and the nuclei are pyknotic or densely stained. Superimposition gives the illusion of large irregular nuclei and multinucleation. The finding of the cuticular membrane, stumps of cilia, and over-all suggestion of being columnar identify these cells as benign. Undifferentiated groups of basal mucosa may cause the same type of confusion with anaplastic or oatcell types, but the regularity in the size of the nucleus and other normal characteristics reveal their true identity. Degenerated forms of the ciliated and other cells should cause no difficulty since the cells maintain their original outlines, and the well-known stages of degeneration are easily recognized.

Cytologic patterns encountered in disease: Composite tabulation of the cytograms in the 1,000 cases when correlated to the proper diagnosis revealed a rather interesting arrangement of cell type frequency which was characteristic of three large groups of diseases:

1. Acute and chronic suppurative bronchopneumonitis with the leukocyte prominent or predominating
2. Acute and chronic allergic bronchopulmonary diseases with the eosinophil present or prominent

3. Neoplastic bronchopulmonary disease with the malignant cell types present

It is important to note that there is nothing characteristic in the cytogram of the first group to identify any one particular disease. All that can be said from any cytogram is that a bronchopulmonary suppurative process is present. Thus, acute bronchitis, bronchopulmonary suppuration, lung abscess, and bronchiectasis are indistinguishable in terms of their cytograms (figure 4). The presence of normal cellular elements usually varies directly with the chronicity. None are found in the chronic cases unless contamination occurs. The presence of mucin in the smears cannot be depended upon diagnostically since a severe bronchitis may have just as intricate a lattice work of mucous strands enmeshing leukocytes as a case of advanced bronchiectasis. Degenerative forms of leukocytes and granular detritus are also present in varying proportions depending upon chronicity as well as on freedom of drainage.

In cases of acute or chronic bronchopulmonary suppurative disease of various types, a peculiar cell which has been called the "Papanicolaou" cell has been found. These cells are described as being relatively small with an elliptical form and an oval pyknotic nucleus (2) (figure 4 *F*). They may appear singly or in compact clusters. They are acidophilic or orangeophilic, and fading of the nucleus is characteristic.

During the study of the cellular patterns in the bronchopulmonary suppurative group, clusters of cells sometimes appeared which were strongly suggestive of neoplastic cells of the epidermoid type (figure 5 *C* and *D*). Such cells were encountered in patients with no roentgenographic or bronchoscopic evidence of malignancy, and observation over a period of years did not reveal malignancy. These cells were usually large, closely grouped together, and rarely were seen alone. The cell bodies, although distinguishable, generally fused with each other. They were not of uniform size and took the orangeophilic stain. The nucleus did not have clear-cut outlines and faded into the cytoplasm. Anisokaryosis was usually present and nuclear detail was not prominent. The size of the cell varied from three to five times that of a leukocyte. The cytoplasm did not possess any distinguishing characteristics but was of a fine homogenous granularity. It may well be that these cells represent clusters of ciliated or other benign cells which have undergone metaplasia and, for want of a better name, have been called *pseudomalignant groups*. One group was encountered which had all the characteristics described above except that a suggestion of ciliation was noted.

In the allergic group the characteristic cell is the eosinophil (figure 5 *A* and *B*), which is frequently and prominently present in asthma; in Löffler's syndrome this cell is predominant. Normal elements are always to be found in these cases unless a suppurative process complicates the underlying disease. Pseudomalignant cell groups such as have been described above are also occasionally found in these diseases.

In the neoplastic group, the cell characteristics of the parent mass were found in varying numbers. Sometimes these cells are obvious and at other times only

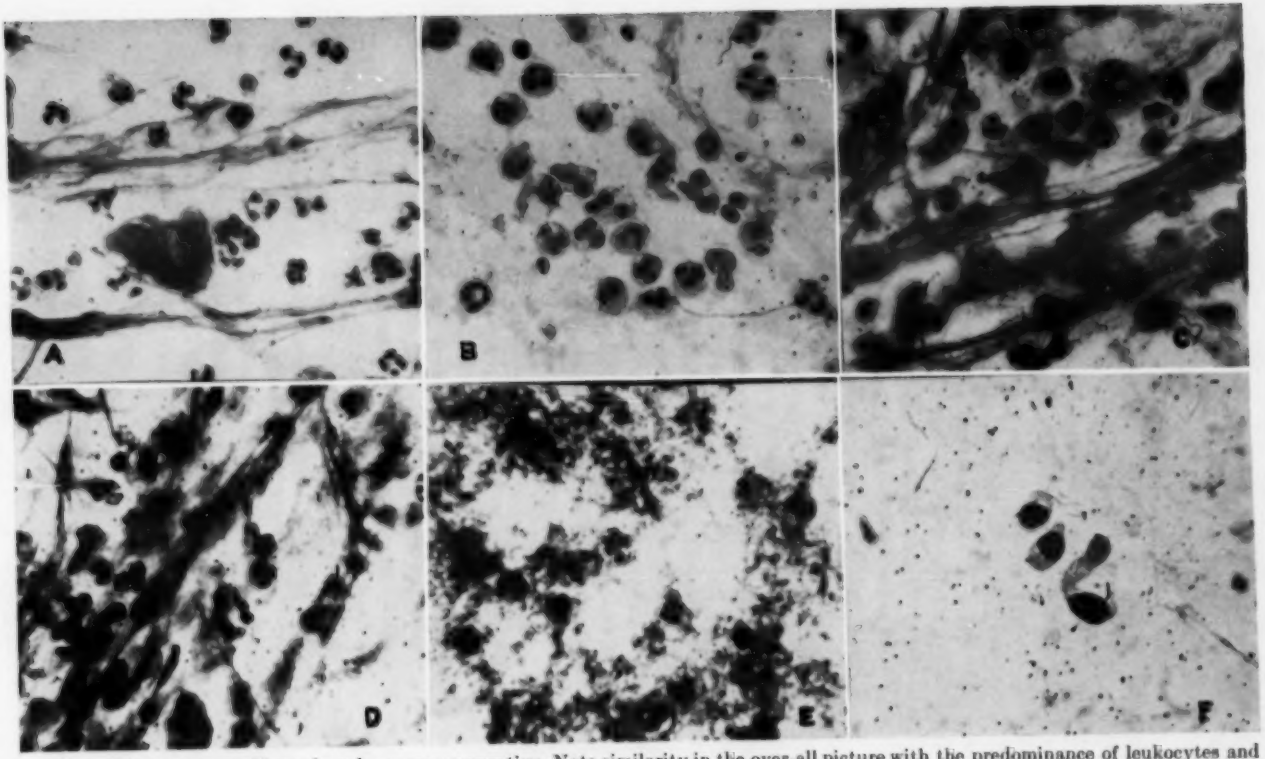

FIG. 4. The cytogram of bronchopulmonary suppuration. Note similarity in the over-all picture with the predominance of leukocytes and the presence of mucin strands.

A. Acute bronchitis.

B. Bronchopulmonary suppuration, obstructive in type.

C. Acute lung abscess.

D. Bronchiectasis, freely draining.

E. Bronchiectasis, poorly draining. Note granular detritus and degenerating forms of leukocytes.

F. "Papanicolaou" cells noted in a case of influenzal pneumonitis. A columnar cell lies below.

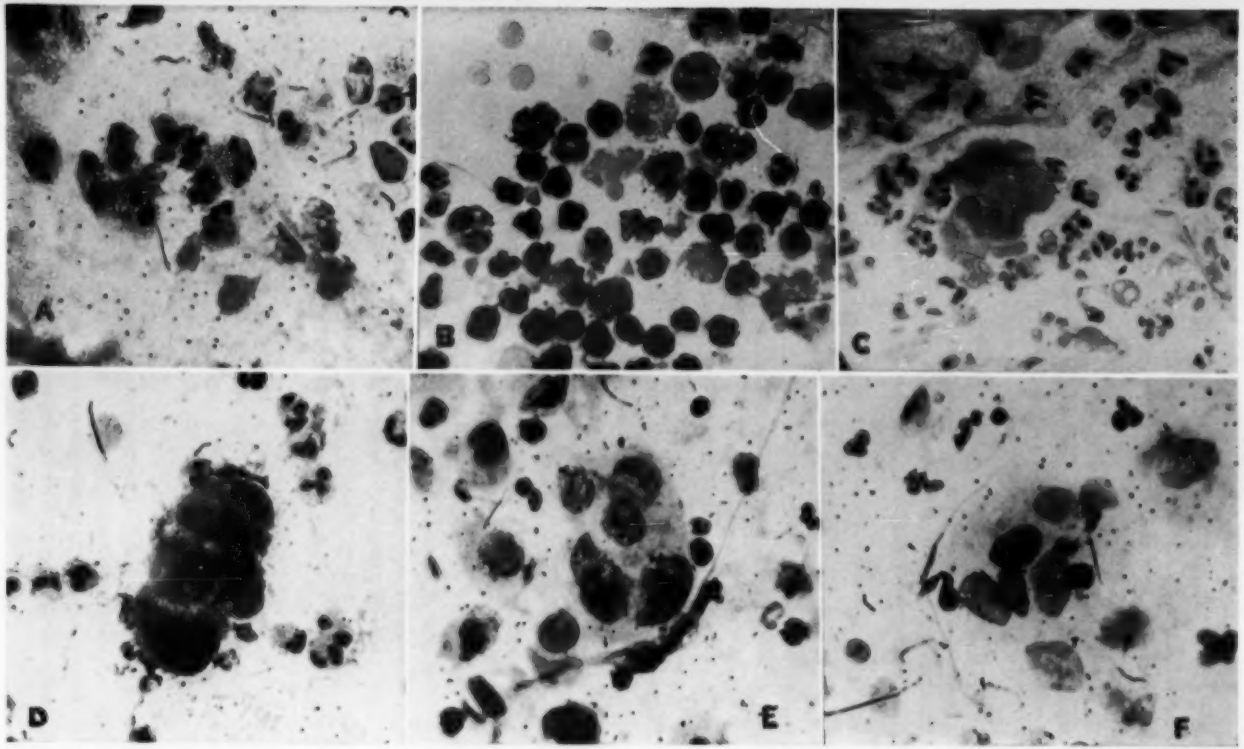

FIG. 5. Cells noted in allergic diseases of the lungs. Pseudomalignant types seen in inflammatory conditions of the lungs and bronchi. Frankly malignant forms.

A. Acute asthmatic crisis showing a group of characteristic eosinophils.

B. Löffler's syndrome in a child of seven years of age. Note high incidence of eosinophils.

C. Large clump of pseudomalignant cells seen in a case of severe bronchopulmonary suppuration. Note poorly defined and anisokaryotic nuclei and orangeophilic cytoplasm with poorly defined cellular boundaries. Compared with figure 3 *E*, which they grossly resemble except for the absence of cilia.

D. Same as *C*.

E. Malignant cells of the epidermoid type. Note bizarre shape of the nuclei, their hyperchromasia, and the anisokaryosis. The cytoplasm is poorly defined and orangeophilic.

F. Same as *E*.

diligent search will reveal them. Observation with low power will show the clusters, and study with high or oil power will point out their peculiar characteristics. The variation of these cells is tremendous, but generally and singularly they have characteristics and peculiarities which identify them as malignant types (2–6).

These neoplastic cells were recognized by their abnormal size, acidophilic or orangeophilic hyperchromasia, vacuolation beyond normal, or the bizarre shape of the cells. The nucleus proper showed enlargement out of proportion to the cytoplasm. The nucleolus was enlarged or increased in number and anisokaryosis was sometimes present in the same group of cells. Mitoses could be seen easily. The nucleus could be noted to fade into the cytoplasm and lose its sharp contours. Cellular multinucleation was present. In general these cells are distinctly different from the usual normal types found and should be studied carefully to see if they meet the criteria of malignancy.

Comment

It must be remembered that, in spite of the fact that this study appears to give a discrete description of three large groups of diseases with what appear to be pathognomonic cytograms for each group, frequent overlapping was noted when some form of suppuration was superimposed on either the allergic or the neoplastic groups. It may be possible to miss the basic diagnosis entirely if only one section of a smear is studied. Suppuration is commonly found as a complication in these diseases. On the other hand the finding of a suppurative cytogram does not preclude the presence of other diseases such as tuberculosis or fungal infections.

Special attention was paid to pulmonary tuberculosis. In the early uncomplicated cases there was nothing characteristic in the cytogram, and only normal cells were noted. Most tuberculous foci studied presented a complicating suppurative process of some type; but none of the cytograms indicated anything characteristic of tuberculosis per se. The diagnosis was usually made by finding the tubercle bacilli in the aspirate or irrigate.

In the cases of acute postoperative atelectasis sufficient time apparently is not present for an outpouring of leukocytes, and the specimen in these cases is fairly free of leukocytes and consists almost entirely of mucin.

Summary

A study of the bronchial aspirate or irrigate in a series of 1,000 cases of bronchopulmonary disease was made by the Papanicolaou technique to determine the presence of pathognomonic cytograms.

This study revealed three large categories of bronchopulmonary diseases which may be said to have a characteristic cytogram: acute and chronic suppurative bronchopneumonitis with the leukocyte prominent or predominating; acute and chronic allergic bronchopulmonary disease with eosinophils present or prominent; neoplastic bronchopulmonary disease with malignant cells.

The cytogram of acute or chronic suppurative bronchopneumonitis may be

superimposed on either the allergic or the neoplastic groups to mask the underlying disease. A peculiar pseudomalignant group of cells easily mistaken for malignant epidermoid types, which is present in the bronchopulmonary suppurative group and occasionally in the allergic group, is described.

Sumario

Los Patrones Citológicos en la Enfermedad Broncopulmonar

A fin de determinar la presencia de citogramas patognomónicos, se hizo con la técnica de Papanicolaou un estudio del aspirado o del lavado bronquial en una serie de 1,000 casos de enfermedad broncopulmonar.

Reveló este estudio tres grandes clases de afecciones broncopulmonares de las que cabe decir que muestran un citograma típico: bronconeumonitis supurativa aguda y crónica con el leucocito sobresaliendo o predominando; enfermedad broncopulmonar alérgica aguda y crónica con eosinófilos presentes o sobresaliendo; enfermedad broncopulmonar neoplásica con células malignas.

El citograma de la bronconeumonitis supurativa aguda o crónica puede sobreponerse al de los rupos ya alérgico o neoplásico y enmascarar la afección subyacente. Descríbese un grupo peculiar de células seudomalignas que es fácil tomar por formas epidermoideas malignas, y que se encuentra en el grupo broncopulmonar supurativo y de vez en cuando en el alérgico.

Resume

Aspects cytologiques dans l'affection broncho-pulmonaire

Une étude des éléments obtenus par aspiration ou par irrigation bronchique dans une série de 1.000 cas d'affections pulmonaires a été effectuée par la méthode de Papanicolaou pour déterminer la présence de cytogrammes pathognomoniques.

Cette étude révéla trois grandes catégories de maladies broncho-pulmonaires reconnues comme présentant un cytogramme caractéristique:—la broncho-pneumonie suppurative chronique aiguë et chronique avec abondance ou prédominance de leucocytes; l'affection broncho-pulmonaire allergique aiguë et chronique avec abondance ou prédominance d'éosinophiles; l'affection broncho-pulmonaire néoplasique avec présence de cellules malignes.

Un cytogramme de broncho-pneumonie aiguë ou chronique peut se superposer à celui caractérisant le groupe allergique ou néoplasique et masquer l'affection sous-jacente. Un groupe particulier de cellules pseudo-malignes, pouvant être aisément confondues avec les types épidermoïdes malins et qui se rencontrent dans le groupe broncho-pulmonaire suppuratif et dans le groupe allergique, est décrit.

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EXPERIENCES WITH BRONCHOGRAPHY USING 3,5-DIIODO-4-PYRIDONE N-ACETIC ACID (DIONOSIL®)[1]

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(Received for publication June 20, 1957)

INTRODUCTION

Bronchography as a diagnostic aid in lung diseases has been available since 1922 when Sicard and Forestier first employed iodized poppy-seed oil. Other chemically similar contrast media have also been used, such as iodized sesame or arachis oils. The disadvantages of these media are delayed lung clearance, the development of lipoid pneumonia or lipoid granulomata, fatal collapse, and iodism.

The ideal bronchographic material should be nonirritating to the lungs and produce no general reaction; it must be miscible with bronchial secretions and eliminated quickly and easily from the lung; it should be capable of filling or outlining the smaller bronchi without filling the alveoli; it must be capable of maintaining filling until roentgenograms can be exposed; and it should be easy to administer and capable of giving consistently diagnostic roentgenograms.

In 1938 aqueous solutions of diodone were first used to overcome the disadvantages of the oily contrast media (1). The results were not wholly satisfactory because the hypertonic solutions used caused instantaneous "flooding" and a fleeting bronchogram. Since 1948, when Fischer (2) described good results with a water-soluble contrast media, the use of such media has increased. Media such as umbradil, Carboxymethylcellulase, umbradil viscous B, Ioduron®, Methocel-Diodrast®, and, most recently, Dionosil®, have become available for use in bronchography. Dionosil is the N-propyl-ester of 3,5-diiodo-4-pyridone N-acetic acid in either peanut oil or methylcellulase, the former known as the oily preparation, the latter the aqueous preparation. The material is hydrolyzed within a few hours of administration, the alcohol is metabolized, and the remaining organic anion is excreted in the urine. There is no liberation of free iodine and therefore no iodine reaction. The aqueous preparation is thought to be more irritating, shows fixation particularly in the lower lobes of the lung, and does not spread as far distally to fill the finer bronchial radicles, but is a more satisfactory preparation than iodized oil (3). The oily Dionosil is less irritating, does not "fix" in any lobe, flows freely, and fills the finer bronchial radicles, but shows frequent alveolar filling. Examination of excised portions of lung in human beings after bronchography with Dionosil aqueous has shown alveolar thickening due to edema and hyperemia, and large histiocytes four days after the bronchographic procedure. Changes persisted up to seven weeks after the oily prepara-

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tion had been used and consisted of free material in the alveoli and alveolar walls, particularly in the abnormal areas of the lung. At seven weeks only an occasional globule was seen in the pulmonary septa and occasionally in a phagocyte. No granulomata were seen after either preparation. Dionosil has been widely used by the British (3–6) with excellent results.

Material

Dionosil has been used as the contrast media at Fitzsimons Army Hospital since November, 1954, and it is the purpose of this paper to summarize the results and reactions of 690 bronchographic procedures on 536 patients.

Method

The patients are premedicated in the usual manner with a barbiturate, codeine, and atropine, two hours before the procedure. The pharynx, epiglottis, and trachea are anesthetized with 2 per cent cocaine, and a soft rubber catheter is inserted, through either the nose or mouth, directly into the trachea. Two per cent cocaine is then sprayed through the catheter to insure good anesthesia at the level of the bifurcation and in the main stem bronchi.

The patients are then brought to the Department of Radiology, and the filling of the bronchi is accomplished under fluoroscopic control. This has been found to be consistently more satisfactory than "blind" filling. The catheter is placed in the side to be studied and the patient is rolled into a lateral position with this side down. As the bronchi begin to fill with Dionosil, the patient's position can be changed slightly, if necessary, to facilitate adequate distribution of the Dionosil to all segments. Using this method, complete and controlled filling of all segments can be accomplished, and it is very seldom that a study has to be repeated because of incomplete visualization of specific bronchi. Spot films are taken in various projections at the time of fluoroscopy, and these are frequently helpful additions to the routine large films, especially in postoperative cases and in those patients with anatomic variations.

Following the roentgenographic procedure the patients are returned to the anesthesia room where postural drainage is carried out for ten minutes, and then they are returned to their respective wards where further postural drainage is performed.

The average amount of contrast media used was 19 ml. per side, but this varied from 8 to as much as 40 ml. per side. The larger amounts were used in patients who were coughing and subsequently swallowing much of the contrast material.

Discussion

Aqueous Dionosil was used in 641 bronchograms and oily Dionosil was used in 49 bronchograms. The patients who received the aqueous media did not cough any more than those who received the oily preparation. Oily Dionosil was noted to flow into the bronchi more rapidly, and in many instances alveolar filling was so great that interpretation was rendered difficult. The finer bronchial radicles did not fill as readily with the aqueous media, but, if more time was allowed during fluoroscopic filling and if the patients were instructed to breathe deeply during the procedure, satisfactory filling did occur. Most of the patients in whom oily Dionosil was used were children who were under general anesthesia, and in these patients the rapid filling was believed to be helpful and only spot film studies were used in these instances. In this situation the bronchi were aspirated through an endotracheal catheter immediately after the film studies.

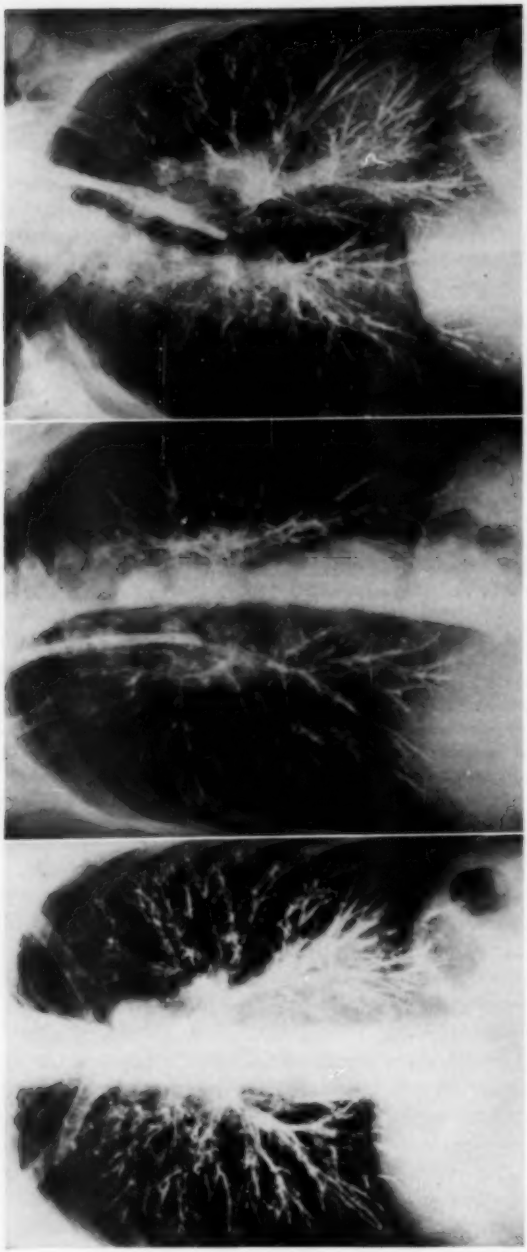

FIG. 1. Postero-anterior and both anterior oblique views of a normal bilateral bronchogram of a 43-year-old woman with hemoptysis. Note the adequate filling of peripheral bronchi without alveolization of the contrast medium.

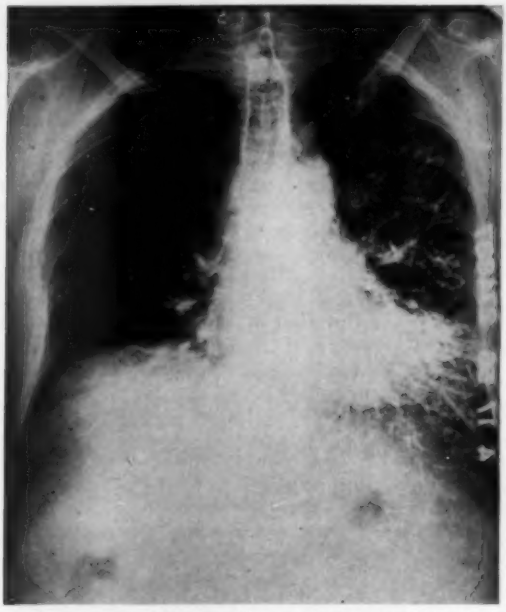

FIG. 2. Left bronchogram of a 36-year-old woman, showing difficulty encountered with Dionosil in oil. Alveolization is rapid and uncontrollable.

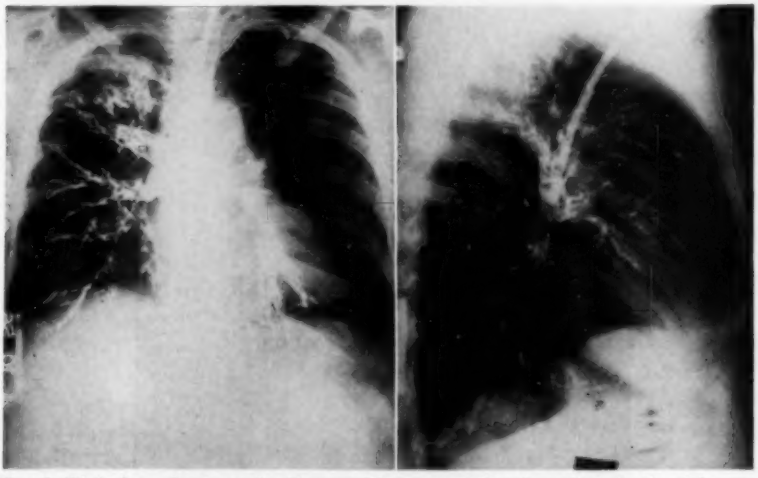

FIG. 3. Right bronchogram of a 40-year-old woman with tuberculosis in the right upper lobe, treated for six months, showing volume loss in posterior and apical segments of the right upper lobe and redistribution of the anterior segment of the right upper and right middle lobe segments. It also shows cylindrical bronchiectasis of involved segments in the right upper lobe.

The large follow-up films on adults were taken with the patients lying prone to prevent rapid loss of media from the upper lobes. By using this technique, adequate bronchograms were obtained in approximately 98 per cent of the cases. An adequate bronchogram is defined as one in which filling of the lobe or segment permits accurate interpretation.

There were 62 reactions in 690 bronchographic procedures, an incidence of approximately 9 per cent. Most of the patients showed a transient hyperpyrexia with fever varying from 99° to 102°F. Some of the patients noted dyspnea and/or wheezing which began four to six hours after the procedure. These symptoms were severe on only six occasions and required medication for relief such as Isuprel®, ephedrine, or an antihistamine preparation. The remainder of the reactions were mild and the duration of the reaction was usually eighteen to twenty-four hours. It is thought that some of these reactions were due to inadequate postural drainage after the procedure, as there have been no serious reactions since more intensive drainage has been instituted. One patient complained of nausea and burning in the chest.

There has been one mild cocaine reaction which responded quickly to intravenous barbiturates, and no serious reactions to the anesthesia have been noted.

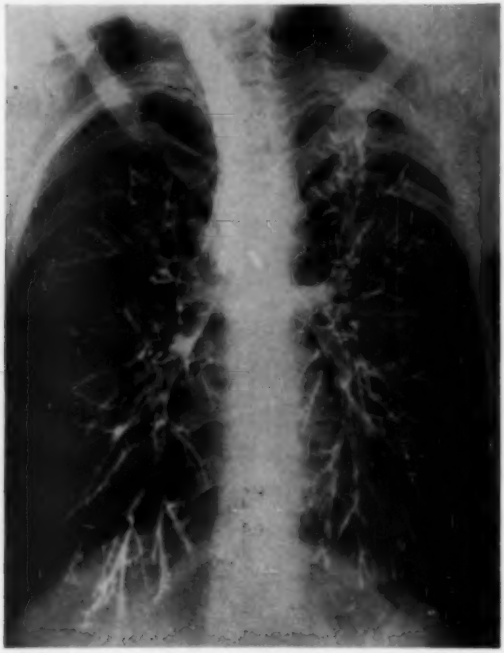

FIG. 4. Postero-anterior view of a bilateral bronchogram of a 37-year-old woman with tuberculosis of the left upper lobe who was treated for eight months with antimicrobial drugs. The bronchogram demonstrates residual bronchiectasis in the anteroposterior segment of the left upper lobe and volume loss due to fibrosis.

One patient was believed to have a hypersensitivity reaction and a brief description follows:

The patient, a 35-year-old white female, had a bronchogram with aqueous Dionosil on March 1, 1955. After approximately 5 ml. of the media had been instilled through the tracheal catheter, the patient became extremely dyspneic and orthopneic. She ran to a window to catch her breath. Physical examination revealed that her blood pressure was normal, the respiratory rate was 60 per minute, and the pulse rate 160 per minute. Auscultation of the lungs revealed sibilant rales and diffuse wheezing throughout both lung fields. The patient was placed immediately on postural drainage and expectorated a small amount of Dionosil. Intramuscular adrenalin (1:1,000 concentration), 5 minims, intravenous aminophylline, 250 mg., and nasal oxygen relieved her symptoms slightly. However, she became apneic for a period of thirty seconds during which time the blood pressure was stable and the pulse rate was 160 per minute. An infusion of 500 ml. of 5 per cent glucose (to which 25 units of corticotropin had been added) was administered. Three hours after the bronchogram had been attempted, the respiratory rate had decreased and the sibilant rales had disappeared. By 10 p.m. the patient was only slightly dyspneic and did not require oxygen. On March 2, 1955, she was asymptomatic, and physical examination of the chest was normal. Corticotropin (Acthar gel®), 20 units, twice daily, was continued for three days and no further reaction occurred.

Many of the patients have a complete study of only one lung at a time and this selection is made on the basis of clinical considerations. Any patient who has limited pulmonary function is believed to be a poor risk for a bilateral bronchogram at one sitting. In 2 instances early in the present series patients with pulmonary emphysema and suspected bronchiectasis developed severe dyspnea after the second side had been filled. One of these patients became mildly cyanotic. In both instances there was a rather poor flow of contrast media into the peripheral bronchi, and some of the trouble was undoubtedly due to obstructed airways. This difficulty in peripheral filling has been noted in other patients with pulmonary emphysema.

Another patient, a 34-year-old white female, had an uneventful bronchogram on the right side demonstrating some minimal cylindrical bronchiectasis and a slight loss of volume in the right upper lobe following pulmonary tuberculosis. Three days later internal spirometric studies were performed, and she had a marked air-flow obstruction on the right. These findings were confirmed at this time by several duplications of identical figures. Eight days later a repeat study was performed and normal readings were obtained. This change was thought to reflect probable residual edema and narrowing of the bronchial structures following the bronchogram.

Summary

The experiences with Dionosil at Fitzsimons Army Hospital are in general agreement with previous reports in the literature. As a result, this medium is used routinely for bronchography at this hospital. It permits repeated bronchograms to be done with little hazard to the patient, and the technical quality of the bronchograms is excellent. The rapid clearing of the opaque material does not interfere with subsequent film studies of the chest. The procedure should be carried out with the smallest amount of radiopaque material possible. Adequate

postural drainage performed following the bronchogram will reduce reactions to the contrast material to a minimum. The importance of an evaluation of pulmonary function prior to bronchography has been stressed, and in those patients with limited pulmonary function it is believed that only one side should be examined at a time.

Sumario

Observaciones con el Uso de Dionosil en la Broncografía

Las observaciones relativas a Dionosil® en el Hospital Militar Fitzsimons convienen en general con las publicadas anteriormente en la literatura. A consecuencia de ello, se usa corrientemente este medio en el hospital. Permite el mismo obtener broncogramas repetidos con poco peligro para el enfermo y la calidad técnica de los broncogramas es excelente. El rápido despejo de la substancia opaca no obstaculiza los subsiguientes estudios radiográficos del tórax. El procedimiento debe llevarse a cabo con la mínima cantidad posible de medio opaco. El adecuado drenaje postural después de la broncografía reducirá al mínimo las reacciones al medio de contraste. Recálcase la importancia que tiene la justipreciación de la función pulmonar antes de la broncografía y opínase que, en los enfermos que tienen limitada la función pulmonar, no debe examinarse más que un lado cada vez.

Resume

Résultats expérimentaux de la bronchographie au Dionosil

Les résultats de l'expérimentation du Dionosil® au Fitzsimons Army Hospital concordent avec ceux des rapports publiés antérieurement. En conséquence, ce milieu est utilisé de façon routinière dans cet hôpital pour la bronchographie. Cette préparation permet la répétition des bronchogrammes sans grand risque pour le malade et la qualité technique du bronchogramme est excellente. L'élimination rapide de la substance opaque évite tout obstacle à l'examen ultérieur des radiogrammes thoraciques. Cette technique doit être appliquée en employant une quantité de substance opaque la plus réduite possible. Le drainage en position adéquate à la suite du bronchogramme réduira au minimum les réactions au milieu de contraste. L'importance d'une évaluation de la fonction pulmonaire avant la bronchographie a déjà été signalée, et les auteurs estiment que chez les sujets dont la fonction pulmonaire est réduite il ne faut explorer qu'un seul côté à la fois.

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ENDOBRONCHIAL TUBERCULOSIS IN CHILDREN[1,2]

A Study of 156 Patients

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(Received for publication May 3, 1957)

Introduction

The association of endobronchial involvement and compression of the trachea and bronchial tree by enlarged tuberculous lymph nodes has been described by many observers, and the clinical syndrome seen in these children has been well known for many years. Pathologic evidence of the encroachment of caseous lymph nodes on the trachea and bronchi of children with primary pulmonary tuberculosis has also been recognized for a long time (1–4). As usually occurs, the early cases reported were those of patients with severe symptoms and fatal outcomes (5,6), but later less serious forms of bronchial involvement were recognized by physical signs and symptoms (7). The association of tuberculous endobronchial involvement in children with segmental or lobar roentgenographic shadows has been repeatedly stressed (8–13). The more frequent use of bronchoscopy has led to a better understanding of the pathogenesis and course of endobronchitis in children (14–16).

In the Chest Clinic of the Children's Medical Service of Bellevue Hospital nearly one thousand consecutive cases of primary pulmonary tuberculosis were observed during the years 1930 through 1946. The mortality rate in the children was slightly over 20 per cent; 95 per cent of the deaths were caused by meningitis, miliary tuberculosis, more protracted forms of hematogenous tuberculosis, or locally progressive primary tuberculosis with evidence of cavitation and bronchogenic spread (17).

Although the clinical picture of "pressure syndrome," due to the encroachment of tuberculous nodes on the tracheobronchial tree, was frequently diagnosed, it was not often directly responsible for the death of a child during this period. One child with tuberculous disease died of suffocation following the extrusion of caseous material from lymph nodes into the trachea, and a few children developed fatal caseous pneumonia secondary to erosion of a caseous node through the bronchial wall. It is evident from the experience in Bellevue Hospital and from the reports of other observers that, prior to antimicrobial therapy, endobronchial tuberculosis was not a frequent cause of death in children with primary tuberculosis.

Many investigators have shown, however, that permanent bronchial or pul-

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[2] Aided by grants from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association; the Division of Research Grants and Fellowships of the National Institutes of Health, Public Health Service; and Parke, Davis and Company.

monary damage frequently follows endobronchial tuberculosis. Most observers have reported a high incidence of bronchiectasis following tuberculous endobronchial involvement in children (18–20). Follow-up studies of the group of children with primary pulmonary tuberculosis seen in Bellevue Hospital before the streptomycin era also produced evidence that bronchiectasis and lesser deformities of the bronchial tree frequently followed tuberculous endobronchitis.

Soon after antituberculous drugs became available, a study of endobronchial disease in children with primary pulmonary tuberculosis was begun in cooperation with the Department of Otolaryngology of the New York University Post-Graduate Medical School. The investigation was designed to study: the diagnosis and course of endobronchial involvement by means of repeated bronchoscopy; the effect of therapy with streptomycin on the local lesion; and the incidence of permanent bronchial and pulmonary changes resulting from endobronchitis.

A preliminary report on this study appeared in 1952 (21). The results of specific therapy were disappointing; no evidence was obtained by bronchoscopy that streptomycin had a measurable beneficial effect on endobronchial involvement due to encroachment of tuberculous nodes on bronchi. In some children the local disease progressed while the patient was receiving specific therapy; rapid disappearance of the roentgenographic shadows representing segmental obstruction rarely occurred. As new antituberculous drugs were made available, it was decided to test their efficacy in continuation of the study. Bronchoscopic evidence of endobronchial involvement was used as the sole criterion for admission to the study group, and duration of the disease was measured by repeated bronchoscopies.

Plan of Investigation

During the years of the study, children in the Chest Clinic of the Children's Medical Service were selected for bronchoscopy if they presented symptoms suggestive of endobronchial involvement or roentgenographic evidence of segmental obstruction. In addition, a small group of children with normal chest films and no symptomatic evidence were bronchoscoped because of repeated cultures of tubercle bacilli from the gastric washings. Obviously this is a selected group, and no claim is made that it includes every child with tuberculous endobronchitis during the period of the study. Some parents refused bronchoscopy; some children were too ill with other forms of tuberculosis, such as meningitis, to be bronchoscoped; in some the diagnosis was undoubtedly overlooked.

With the purpose of avoiding unnecessary psychic and physical trauma due to repeated bronchoscopies, a technique of anesthesia was developed by the departments of Otolaryngology and Anesthesiology (22). Rectal anesthesia with sodium Surital®, 1 gm. per 50 pounds of body weight, was used together with topical anesthesia of the entire tracheobronchial tree with 0.5 per cent aerosol tetracaine hydrochloride (Pontocaine®). This type of anesthesia permitted the insertion of the bronchoscope while the child slept and reduced struggling, coughing, and laryngo- or bronchospasm to a minimum. Moreover, it allowed the bronchoscopist time to do a careful and detailed inspection of the tracheobronchial tree. Since no inhalation anesthetic was used, it was possible to recognize the slight changes in the mucous membrane now known to be associated with early endobronchial involvement.

From 1947 through July, 1954, 156 children were found to have bronchoscopic evidence of tuberculous endobronchitis. These patients form the basis of the present report.

Observations

During the years of the study, about 65 per cent of the total ward population of tuberculous patients was less than four years of age, but 83 per cent of the patients with proved endobronchial involvement were less than four years of age. Boys and girls were about equally represented in the ward population, but there were 94 males to 60 females in the group with proved endobronchitis. Cough was the most frequent symptom but often was absent in the older group. Severe symptoms such as wheeze, dyspnea, cyanosis, and marked spasmodic cough were usual in the young infant (table 1).

All patients had three gastric lavages soon after admission and thereafter at least once every month while in the hospital. Cultures of bronchial fluid were obtained from each endoscopic examination.

It is obvious from table 2 that gastric lavage was more valuable in the diagnosis of tuberculosis in children than culture of bronchial fluid even when active endobronchitis was present.

Roentgenographic evidence of bronchial obstruction was seen in all but 15 of the 156 patients (table 3). The localization of obstruction according to segments is shown in figure 1. Evidence of obstruction was seen more often in the

TABLE 1

Distribution of 156 Children with Tuberculous Endobronchitis by Age and Symptoms

Age	Total Number	With Definite Symptoms	
		Number	Per Cent
<6 months	10	9	90
6–11 months	29	18	62
1–2 years	72	26	36
3–4 years	25	4	16
>5 years	20	1	5
Totals	156	58	37

TABLE 2

One-hundred and Fifty-six Patients with Tuberculous Endobronchitis

Cultures for Tubercle bacilli from Gastric and Bronchial Fluids

Positive GF* Negative BF†	Negative GF Positive BF	Positive GF Positive BF	Negative GF Negative BF
59	8	37	52

Patients with positive cultures—104 66.7%
Patients with positive GF cultures—96 61.5%
Patients with positive BF cultures—45 28.1%

* GF—gastric fluid
† BF—bronchial fluid

TABLE 3

ROENTGENOGRAPHIC EVIDENCE OF BRONCHIAL OBSTRUCTION

Limited to one or more segments of one lobe	110
Two or more lobes	27
Entire lung	2
Bilateral	7
Emphysema only	4
No evidence	15
Total	156

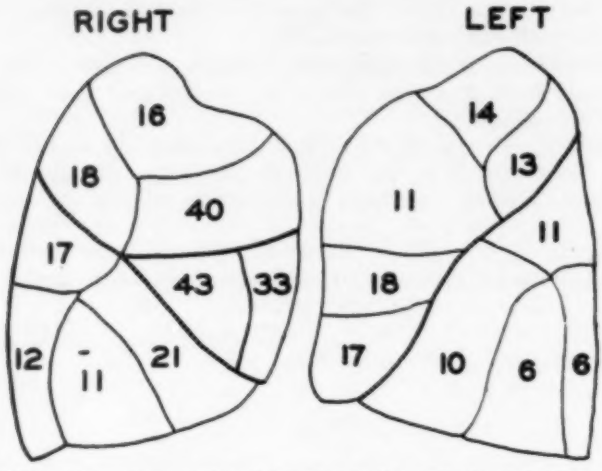

FIG. 1. Distribution of segmental obstruction in 156 patients with tuberculous endobronchitis.

right lung, most frequently in the middle lobe and in the anterior segment of the right upper lobe. On the left side the lingula was most often involved.

The difficulty of assessing duration of obstructive lesions by roentgenography was so great that no tabulation has been attempted. In the majority of cases the primary focus was in the same segment or lobe as the obstructed area, as shown by the later finding of a characteristic calcified primary focus. The diagnosis of obstruction in such cases was made by the typical triangular shape of the shadow, by increases or decreases in size of the roentgenographic shadow not usual in primary infections, by the bronchoscopic evidence of associated endobronchial involvement in the bronchus of the segment under question, or by later evidence of progressive decrease in the size of the involved lung area. In the cases in which the primary lesion was definitely in another segment or lobe, or in the patients who had two or more segments or lobes involved, the duration of obstruction was less difficult to assess.

Antimicrobial therapy: The impossibility of doing a controlled study from the

available clinical material was apparent from the beginning. Cases for treatment could not be selected at random since many of the children with bronchoscopic evidence of bronchial involvement were already under treatment for miliary tuberculosis, meningitis, or other forms of progressive disease requiring treatment. Other children developed complications for which therapy was mandatory. In addition, it seemed advisable to treat all patients, usually young infants, who had severe symptoms, especially marked paroxysmal cough associated with cyanosis. One hundred and twenty of the 156 patients received specific therapy.

During the period of the study there were many changes in the regimen of specific therapy. At first streptomycin was given for only six weeks, often as the sole therapeutic agent. Later many patients received streptomycin for six or more months, combined with PAS or sulfone Promizole®. During the last two and a half years of the investigation, isoniazid combined with PAS or sulfone was given, usually for at least a year. Cycloserine was tried in a few patients who had not responded to other forms of therapy. Throughout the years of the study, however, all patients who had serious forms of tuberculosis, such as meningitis or miliary tuberculosis, received specific therapy for one to three years. The distribution of patients according to duration of therapy is shown in table 4.

Bronchoscopic findings: All of the children included in this study had definite bronchoscopic evidence of endobronchitis. The findings presented in table 5 represent the total count of each type of abnormality, and a single case may be represented in more than one category. Erosion of the bronchial wall was included only if an actual break was seen in the mucosa or if caseous material was seen extruding from the bronchial wall.

One of the first objects of the study was to determine the duration of endo-

TABLE 4

ANTIMICROBIAL THERAPY IN 156 PATIENTS

No specific therapy	36
Antimicrobial therapy for less than 6 months	41
Antimicrobial therapy 6 months to 1 year	22
Antimicrobial therapy 1 year or more	57
Total	156

TABLE 5

BRONCHOSCOPIC FINDINGS IN 156 PATIENTS

Narrowing of lumen due to external compression		36
Narrowing of lumen due to disease within bronchus		42
Stenosis of bronchus		10
Granulation tissue		114
Polyps	54	
Ulceration of bronchial wall		20
Caseous material within bronchus		50
Erosion of bronchial wall		25

TABLE 6

DURATION OF ACTIVE ENDOBRONCHITIS BY BRONCHOSCOPY IN 70 PATIENTS
Relation to Specific Therapy

Duration of Endobronchial Involvement	Number of Patients	Duration of Specific Therapy			
		None	Less than 6 months	6-11 months	More than 1 Year
Less than 3 months	11	4	0	5	2
6-11 months	36	3	12	10	11
More than 1 year	23	3	8	2	10
Total	70	10	20	17	23

bronchial involvement in the group by measuring the interval between the first diagnostic bronchoscopy and healing. The plan was to bronchoscope at least every three months until an end point was reached. Because of the unavoidable delays between bronchoscopies, it has not been possible to calculate the duration of active disease in all patients, but only in 70 children, as shown in table 6.

The investigators believe that in this group of 70 patients in whom the duration of endobronchitis can be measured, no definite beneficial effect of antimicrobial therapy on the endobronchial lesions can be demonstrated. Forty of the 70 children received antimicrobial therapy for six months or more; yet in 33 of these patients active endobronchitis continued to be seen through the bronchoscope for a period ranging from six months after treatment was begun to more than three years later. Moreover, in some children the local disease progressed while the patient was receiving treatment.

As specific treatment apparently does not promote rapid healing of endobronchial involvement, it is important to know whether therapy prevents the irreversible changes in bronchi and parenchyma which were demonstrated so often before treatment was available. A careful roentgenographic follow-up, including bronchography, is necessary to demonstrate the true incidence of damage to lung and bronchial tissues. It is impossible to assess damage only by clinical methods since children with bronchiectasis secondary to tuberculous endobronchitis rarely present the classical picture of suppurative pulmonary disease and may be entirely free from symptoms and signs.

Roentgenographic findings: Thirty-one, or about 20 per cent, of the entire group of 156 patients had roentgenographic evidence of one or more contracted pulmonary segments. Areas of increased radiolucency were not infrequently demonstrated within the areas of clouding.

Bronchographic findings: Bronchography yielded more evidence of damage to bronchi and parenchyma than the usual roentgenograms. Bronchograms were obtained without selection in 103 of the 156 patients, with the results shown in table 7.

In the group with minimal abnormalities the dilatation of the bronchi was

TABLE 7

BRONCHOGRAPHIC FINDINGS IN 103 PATIENTS

Normal		33
Abnormal		70
Minimal abnormalities	20	
Crowding of bronchi	6	
Narrowing of bronchi	4	
Cylindrical bronchiectasis	9	
Saccular bronchiectasis	1	
Marked abnormalities	50	
Crowding of bronchi	5	
Stenosis of bronchi	10*	
Cylindrical bronchiectasis	29†	
Saccular bronchiectasis	6‡	
Total		103

* One of these showed bronchiectasis in resected lobe.

† One additional case showed bronchiectasis in resected lobe.

‡ One additional case showed bronchiectasis in resected lobe.

TABLE 8

RELATIONSHIP OF BRONCHOGRAPHIC CHANGES TO SPECIFIC THERAPY

	Total		Untreated		Treated Less than 6 Months		Treated more than 6 Months	
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Normal bronchograms	33	32.0	6	28.6	11	34.4	16	32.0
Abnormal bronchograms	70	68.0	15	71.4	21	65.6	34	68.0

either slight in extent or localized to a very small area; the bronchial narrowing was never more than 50 per cent.

Many of the bronchograms, especially of those in the group with marked abnormalities, showed two or more deviations from normal. In each case, however, only one reading has been listed for each patient. Usually the abnormalities in the bronchogram were in the segment or lobe originally obstructed.

An attempt has been made to relate the findings on bronchography to antimicrobial therapy (table 8).

Among 103 children with endobronchial tuberculosis, subsequent bronchograms were normal for 33 children (32 per cent) and abnormal for 70 children (68 per cent). As seen in table 8, the frequency of abnormal bronchograms was essentially the same whether the children were untreated, treated for less than six months, or treated for more than six months. Thus, in this group of cases there was no evidence that specific therapy prevented secondary damage to the lungs or bronchi in patients with tuberculous endobronchitis.

Every one of the 50 patients who showed marked changes in bronchograms

TABLE 9

Surgery in 10 Children Following Tuberculous Endobronchitis

Patient	Age on Admission	Year of Diagnosis	Year of Operation	Duration of Endobronchitis	Specific Therapy	Bronchogram	Operative Procedure	Pathology	Bases of Decision for Surgery
1. DA	17 months	1949	1951	3 months, granulations and erosion 3 months, stricture LMB at 4 months.	Daily SM, 4½ months.	Not done	Pneumonectomy, left	Marked saccular bronchiectasis LLL. No evidence of tuberculosis.	Failure to dilate stricture of LMB and marked bronchiectasis LLL.
2. SW	11 months	1949	1953	Polyps 18 months, negative 2 years.	Daily SM and sulfone (Promizole®) 8 months	Not done	Resection, RUL	Contracted RUL, polyp within cavity or dilated bronchus. No evidence of tuberculosis.	Persistent cavity RUL, confirmed by tomogram.
3. FN*	3 months	1950	1953	7 weeks.	Daily SM and sulfone 4½ months.	Saccular bronchiectasis anterior segment RUL (#3).	Attempted resection RUL, adhesions too dense, not completed.	—	Extensive bronchiectasis RUL.
4. LJ	11 years	1952	1953	More than 7 months, erosion into LLLB, no negative bronchoscopy	Daily SM and sulfone 2 months, SM and PAS 3 months.	Not done	Resection superior segment LLL (#6)	Peribronchial thickening, fibro-caseous tuberculosis.	Marked narrowing LUL bronchus, persistence of primary shadow on roentgenogram in adolescent girl.
5. DB	3 years	1950	1955	More than 5 months, no negative bronchoscopy.	Not treated	Saccular bronchiectasis superior segment LLL (#6)	Resection superior segment LLL (#6)	Saccular bronchiectasis (#6) LLL. No evidence of tuberculosis, negative culture.	Contracted (#6) LLL with marked saccular bronchiectasis. Positive culture gastric 5 years after primary tuberculosis.

6. ES†	3 years	1947	1955	More than 3 months, healed 5 months.	Daily SM, 4 months.	Stenosis, RMLB	Resection RML. RLL also resected because of damage to artery due to dense adhesions.	Contracted RML, cylindrical bronchiectasis RML, normal RLL, no evidence of tuberculosis.	Contracted RML and stenosis RMLB.
7. PP	19 months	1949	1955	More than 9 months, no negative bronchoscopy	Daily DHSM and PAS, 6 weeks.	Cylindrical bronchiectasis, RML, crowded bronchi, RML and RLL.	Resection RML. RLL also removed because of dense adhesions.	Cylindrical bronchiectasis RML, normal RLL.	Cylindrical bronchiectasis RML going on to distortion RML and RLL on roentgenogram.
8. EV	4 years	1953	1955	More than 3 months, negative 15 months.	Not treated	Cylindrical bronchiectasis, anterior mediastinal basal segment LLL (#8) and inferior segment LUL (#5).	Resection lingula and anterior mediastinal basal segment, LLL (#8)	Segments airless, minimal bronchiectasis and caseo-calcific primary tuberculosis in each.	Marked cylindrical bronchiectasis lingula and LLL (#8)
9. DM	17 months	1953	1956	Unknown, first bronchoscopy, polyp and granulations, second bronchoscopy, 21 months later, negative.	INH and PAS, 18 months	Narrowing LLLB, cylindrical bronchiectasis, superior and posterior basal segment, LLL (#6, 10)	Pneumonectomy, left	Fibrosis, bronchiectasis. Negative for tubercle bacilli. No cavity found.	Persistent highlight in LLL (#6)
10. MS‡	18 months	1950	1956	More than 3 months, stricture 11 months.	Untreated except for ACTH, 3 weeks, 1951.	Stenosis superior segment LLL (#6). Marked calcification.	Resection LLL	Conglomerate fibrocalcific lesions; scattered granulomata with necrotic centers; tubercle bacilli cultured from specimen.	Large partially calcified area in segment (#6) LLL with stricture of segmental bronchus.

LMB—left main bronchus; LLLB—left lower lobe bronchus; RUL—right upper lobe; LLL—left lower lobe; RML—right middle lobe; RLL—right lower lobe; RMLB—right middle lobe bronchus.

SM—streptomycin; PAS—para-aminosalicylic acid; DHSM—dihydrostreptomycin; INH—isoniazid; ACTH—corticotropin.

* Figure 7. † Figure 5. ‡ Figure 8.

has been studied to learn whether surgical resection of the involved area is feasible or advisable. Up to the present, 10 children have been submitted to surgery, and resection is contemplated in at least 5 more children. Some details of the cases already submitted to surgery are shown in table 9.

Discussion

The clinical, roentgenographic, and bronchoscopic findings in this group of children with tuberculous endobronchitis are very similar to those found by many other observers. The fact is generally accepted that children with pulmonary primary tuberculosis frequently develop tuberculous lesions in the bronchi as a result of encroachment of nodes. Most clinicians agree that symptoms such as cough and respiratory distress are most frequently found in infants and may be absent in older children. This finding is probably explained by the fact that the bronchi are smaller and more easily compressible in the very young child. Observers also agree that a fatal outcome of primary tuberculosis due to bronchial involvement is not common at any age, but is more frequent in infants.

The diagnosis of tuberculous endobronchitis can often be inferred even in the absence of symptoms, since roentgenographic evidence of an obstructed segment or lobe is so frequently found. However, endoscopy is probably the most rewarding of all diagnostic measures. The course of the endobronchial lesion can be observed only by repeated examinations, and such observations are essential to appraisal of response to therapy.

The preliminary report of the present investigation furnished no evidence that antimicrobial therapy had a beneficial effect on tuberculous endobronchitis comparable to its effect on other forms of tuberculosis. This finding has now been confirmed in a larger group of patients, most of whom received therapy for longer periods of time than the original group.

This lack of definite and rapid response to therapy was at first surprising in view of the favorable reports about the treatment of tuberculous endobronchitis in adults. However, a review of the pathogenesis of bronchial tuberculosis in children and adults readily explains the difference in results of therapy.

Tuberculosis of the bronchus in adults is usually due to contamination of the mucosal lining of a bronchus by sputum containing large numbers of tubercle bacilli, usually derived from an adjacent cavity. The ulceration begins in the mucosa and may heal rapidly under specific therapy. Moreover, the antimicrobial agents usually are effective in reducing the amounts of sputum and number of tubercle bacilli in the adjacent cavity so that relapse or reinfection is prevented.

In children, tuberculous endobronchitis is due to encroachment of enlarged nodes on the trachea or bronchi. The nodes become fixed to the bronchial walls by inflammatory changes, and the infection progresses through the walls of the bronchi to the mucosal lining, ultimately creating the specific ulceration or granulation tissue by which a positive diagnosis may be made by the bronchoscopist. The granulation tissue may ultimately form polyps which may persist for months and often recur if surgical removal is attempted. Not infrequently a caseous node erodes the bronchial wall and forms a sinus through which caseous material is extruded.

The basic pathology in this form of endobronchial involvement is thus in the lymph node and there is some reason to believe that tuberculosis of lymph nodes

is less readily susceptible to antimicrobial agents than is the case with tuberculous lesions in certain other tissues. Moreover, in most instances, the enlarged lymph nodes contain considerable areas of caseation, a condition which is presumably unaffected by antimicrobial therapy. These factors, together with the massive involvement of all layers of the bronchial wall readily explain the lack of dramatic response to specific therapy of tuberculous endobronchitis in children, as compared with that obtained in the treatment of bronchial lesions in adults. In a few instances in older children on the tuberculosis ward, where the pathogenesis of endobronchial involvement was similar to that of adults, rapid response to therapy was obtained.

Most clinicians agree on the lack of evidence that antimicrobial therapy has a favorable influence on endobronchitis associated with primary tuberculosis in children (23–25). Certainly new antimicrobial agents and new methods of treatment should continue to be investigated. The use of steroid therapy must be explored further because, in theory, it should have a good effect in the early stages of tuberculous endobronchitis. Steroid therapy might be of less value after caseation or erosion has taken place. Surgical repair of fistulae and removal of caseous nodes during the active phase of tuberculous bronchitis have been attempted (26–28). Until better results are obtained from medical treatment the surgical approach to therapy for severe cases of tuberculous endobronchitis should be considered.

The desire to shorten the course of tuberculous endobronchitis is not prompted by the possibility of a fatal outcome since, as previously noted, such events are rare, but by the fear of the many sequelae of this form of tuberculosis. Deformities of the bronchi, narrowing or even stenosis secondary to healing of granulomatous lesions or fistulae, dilatation of the bronchial walls due to infection and mechanical factors not infrequently follow endobronchial involvement. In addition, there may be parenchymal changes such as fibrosis, associated with prolonged segmental obstructions and damage to the bronchi. The frequency with which such sequelae are reported varies. Where the incidence of serious sequelae is low, the patients are usually not in a hospital group, but are largely asymptomatic children attending outpatient clinics. In spite of the fact that 77 per cent of the children with abnormal bronchograms received specific therapy, the percentage of bronchiectasis in the present series is almost identical with that reported by Jones and associates (18) in an untreated group in 1946.

The indication for surgical removal of tissue showing irreversible damage following tuberculous endobronchitis poses a difficult question which must be considered individually for each patient and always after joint consultation of physician and surgeon. Without surgery there is always the possibility of development of suppurative disease in the damaged area or of a hemorrhage arising from a bronchiectatic focus. These conditions rarely occur during childhood and there are no statistics available from which the risk of developing late symptoms can be computed. If surgical intervention seems advisable, resection during childhood before the lungs have stopped growing is highly recommended, as it avoids all question of overdistension of the remaining lung and of space-filling procedures. Even in spite of careful preoperative study, normal lung tissue sometimes has to be resected because of technical difficulties, but every effort should be made to limit the resection to severely damaged areas.

There would seem to be some types of cases in which surgery always should be considered. These include patients with extensive areas of bronchiectasis, especially in the middle or lower lobes, and obviously airless segments, especially in the right middle lobe or lingula of the left upper lobe or in the apical segment of either lower lobe. All of these have been frequently reported as the site of the so-called "middle lobe syndrome" in adults. Definite marked narrowing or stenosis of a bronchus should also be an indication for surgical consideration, especially when it involves major bronchi. The finding of a large amount of calcium within an obstructed area is one of the clearest indications for surgical intervention. Such areas when resected usually contain caseation and evidences of active tuberculosis. The tuberculosis may be due to local spread of the primary focus, since the normal lymphatic drainage is impeded by collapse of the segment or lobe, or it may be the result of bronchogenic spread from the endobronchitis.

The fact that the conclusions of the investigation here reported are that antimicrobial therapy apparently does not shorten the course of endobronchial involvement in children nor diminish the incidence of sequelae should not be interpreted to mean that specific therapy is contraindicated. There is certainly justification for treating children with tuberculous endobronchitis in the hope of diminishing the dangers of bronchogenic spread.

Summary

A group of 156 children with primary tuberculosis showing endoscopic evidence of bronchial tuberculosis is reported. In this series of of cases, endobronchial tuberculosis was more frequent in children less than four years of age, and in boys. Severe symptoms were more often observed in young infants.

Roentgenographic evidence of obstruction was observed in 90 per cent of the patients. Evidence of segmental obstruction was more commonly seen in the right lung and most often in the right middle lobe and in the anterior segment of the right upper lobe. Seventy-seven per cent of the group received antimicrobial therapy; in 66 per cent of those who were treated, the medication was given for more than six months; in 48 per cent, for more than a year.

Granulation tissue was the most frequent finding on bronchoscopy. Fifty-four of the 156 patients had polyps, and erosion of the bronchial wall was diagnosed in 25 cases. The duration of endobronchial involvement by bronchoscopy could be measured in only 70 patients. It continued for less than three months in 11 children, 4 of whom were untreated; from one to more than three years in 23 children, 18 of whom received specific therapy, 10 for more than a year.

Bronchograms were done on 103 patients without selection and showed abnormal findings in 70, marked abnormalities in 50. Bronchiectasis was the most common evidence of damage, occurring in 70 per cent of those with marked abnormalities. The percentage of children with abnormal bronchograms was very similar in the treated and untreated groups.

There is no evidence that antimicrobial therapy markedly shortens the course of tuberculous endobronchitis due to encroachment of caseous nodes on bronchi. There is also no evidence that the use of specific therapy diminishes the incidence of sequelae in bronchi and parenchyma. Nevertheless, there is justification for treating children with tuberculous endobronchitis in the hope of diminishing the dangers of bronchogenic spread of the tuberculosis.

Sumario

La Tuberculosis Endobronquial en los Niños. Estudio de 156 Enfermos

Preséntase un grupo de 156 niños que tenían tuberculosis primaria y revelaban signos de tuberculosis bronquial. En esta serie de casos, la tuberculosis endobronquial fué más frecuente en los niños de menos de cuatro años de edad, y en los varones. Se observaron síntomas graves más a menudo en los lactantes pequeños.

En 90 por ciento de los enfermos, se observaron signos radiográficos de oclusión. Signos de oclusión segmental fueron notados más comúnmente en el pulmón derecho y más a menudo en el lóbulo medio derecho y en el segmento anterior del lóbulo superior derecho. Setenta y siete por ciento del grupo recibieron terapéutica antimicrobiana; en 66 por ciento de los tratados, se administró la medicación más de seis meses; en 48 por ciento, más de un año.

El tejido de granulación fué el hallazgo más frecuente con la broncoscopia. Cincuenta y cuatro de los 156 pacientes tenían pólipos y en 25 casos se diagnosticó erosión de la pared bronquial. Con la broncoscopia no pudo medirse más que en 70 enfermos la duración de la invasión endobronquial. Continuó menos de tres meses en 11 niños, 4 de los cuales no fueron tratados; de un año a más de tres años en 23 niños, 18 de los cuales recibieron terapéutica específica, 10 de ellos por más de un año.

Se hicieron broncogramas en 103 enfermos sin seleccionar, mostrando hallazgos anómalos en 70, notables anomalías en 50. La bronquiectasia fué el signo más común de lesión, observándose en 70 por ciento de los que tenían anomalías notables. El porcentaje de niños que revelaron broncogramas anómalos fué muy semejante en los grupos tratado y no tratado.

No hubo pruebas de que la terapéutica antimicrobiana acortara notablemente la evolución de la endobronquitis tuberculosa debida a intrusión de ganglios caseosos en los bronquios. Tampoco hay pruebas de que el uso de la terapéutica específica disminuya la incidencia de secuelas en los bronquios y el parénquima. Sin embargo, hay justificación para tratar a los niños que tienen endobronquitis tuberculosa con la esperanza de atenuar los riesgos de la difusión broncógena.

Resume

Tuberculose endobronchique chez l'enfant: Etude comprenant 156 patients

Rapport relatif à un groupe de 156 enfants tuberculeux présentant une primo-infection et des signes endoscopiques de tuberculose bronchique. Dans cette série de cas, la tuberculose endobronchique était plus fréquente chez les enfants âgés de moins de 4 ans et chez les garçons. Les symptômes graves s'observaient plus souvent dans le premier âge.

Des signes radiologique d'obstruction étaient visibles chez 90% des malades. L'obstruction segmentaire était généralement rencontrée dans le poumon droit et plus souvent dans le lobe moyen droit et dans le segment antérieur du lobe supérieur droit. Dans ce groupe, 77% des malades étaient soumis à la thérapeutique antibactérienne; 66% des cas traités reçurent la médication pendant plus de 6 mois et 48% pendant plus d'un an. Le signe le plus souvent observé en bronchographie était la granulation tuberculeuse. Sur 156 malades, 54 avaient des polypes et dans 25 cas il existait une érosion de la paroi bronchique. La durée de la lésion endobronchique n'a pu être déterminée par la bronchoscopie que chez 70 patients. Cette lésion a persisté moins de trois mois chez 11 enfants, dont 4 non traités; de 1 à plus de 3 ans chez 23 enfants, 18 d'entre eux étant soumis à une thérapeutique spécifique, 10 de ces derniers pendant plus d'une année.

Les bronchogrammes furent effectués chez 103 malades non-selectionnés, 70 révélaient des signes anormaux et 50 des altérations marquées. La lésion la plus commune était la bronchiectasie, celle-ci était décelée dans 70% des cas présentant des lésions marquées. Parmi les enfants dont les bronchogrammes étaient anormaux, le pourcentage était à peu près semblable dans les groupes traités ou non traités.

Il n'est pas démontré que la thérapeutique antibactérienne abrège de façon marquée le cours de la tuberculose endobronchique due à l'envahissement des bronches par les ganglions caséeux. Il n'y a également aucune preuve indiquant que l'emploi d'une thérapeutique spécifique diminue l'incidence des séquelles au niveau des bronches et du parenchyme pul-

monaire. Néanmoins, dans l'espoir de réduire le danger de dissémination bronchogène, le traitement de la tuberculose endobronchique de l'enfance est justifié.

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CASES ILLUSTRATING SEQUELAE OF TUBERCULOUS ENDOBRONCHITIS

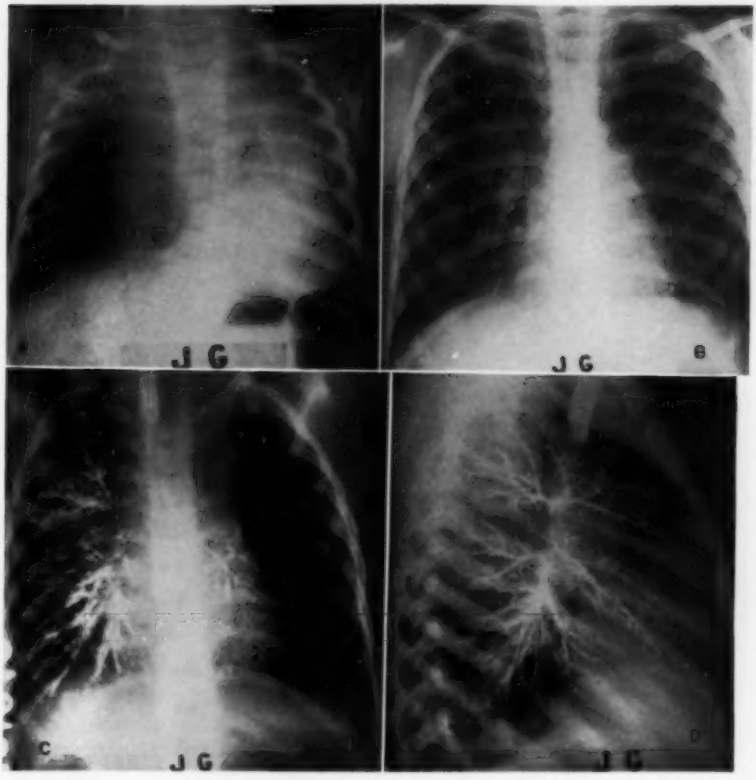

FIG. 2 (J.G.). *Negative bronchogram.* Age, six months. Bronchoscopy of July 28, 1949, showed compression and ulceration of medial surface of right main bronchus; duration to healing six months. Dihydrostreptomycin and PAS, three months.

A. Roentgenogram obtained on June 7, 1949, showing mediastinum and heart shifted markedly to left; clouding of right upper lung field to third anterior rib; increased aeration in remaining right lung field.

B. Roentgenogram obtained on August 8, 1956, showing essentially normal lung fields.

C. and *D.* Negative bronchograms of right lung, obtained on September 5, 1956.

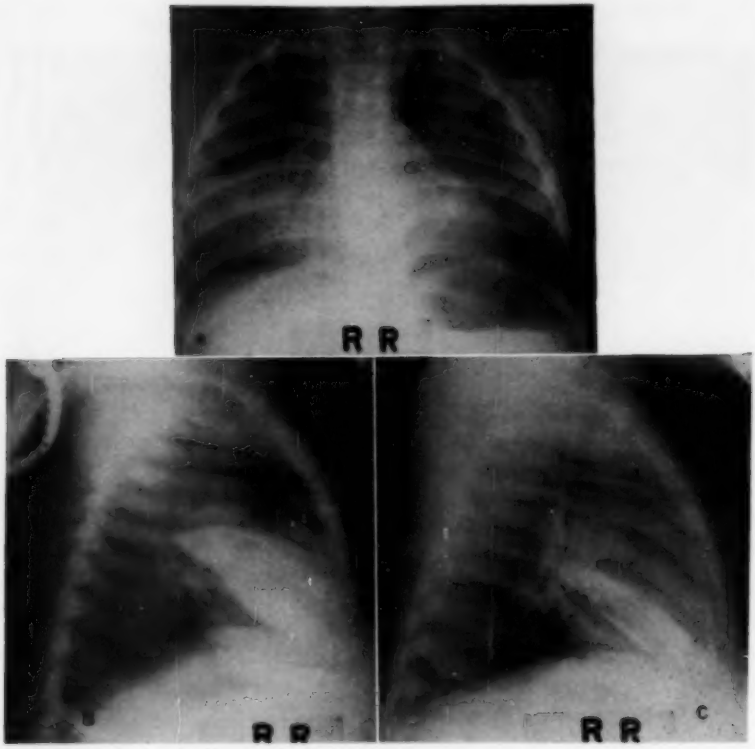

FIG. 3 (R.R.). *Marked crowding of bronchi.* Age, eight months. Bronchoscopy of April 24, 1952, showed granulation tissue in the right middle lobe orifice; duration, seven months. End result, marked narrowing of right middle lobe orifice. Isoniazid and PAS, six months.

A. Roentgenogram of April 16, 1952, showing area of triangular clouding below the sixth posterior rib on the right.

B. Right lateral view, showing triangular clouding located in the right middle lobe.

C. Right lateral view obtained on June 25, 1952, showing marked diminution in the size of the right middle lobe shadow.

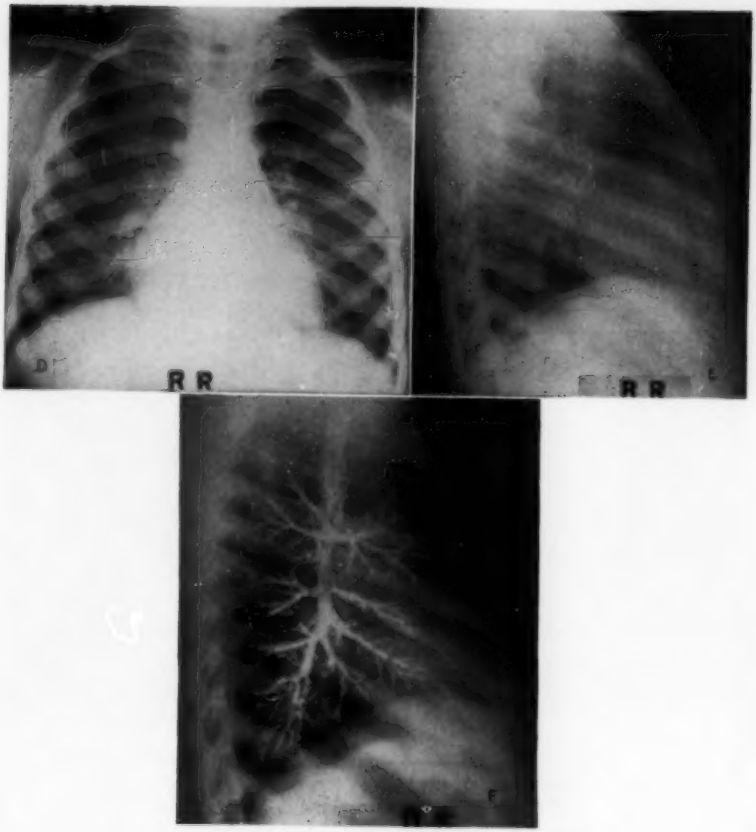

FIG. 3-*D-F*

D. and *E*. Postero-anterior and lateral views obtained on September 9, 1956; essentially negative.

F. Right lateral bronchogram showing crowding of bronchi of right middle lobe.

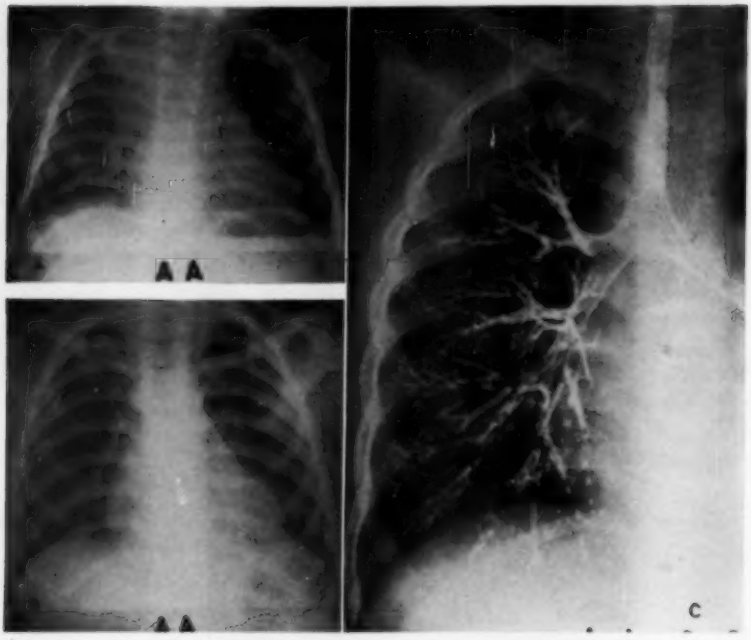

FIG. 4 (A. A.). *Marked bronchial narrowing.* Age, seven months. Bronchoscopy on November 19, 1953, showed granulation tissue of the right upper lobe bronchus. A repeat bronchoscopy on December 9, 1954, showed narrowing of the right upper lobe orifice to pinpoint; mucosa, inflamed.

A. Roentgenogram of May 29, 1953, showing clouding of inner half of right upper lung field continuous with hilar shadow.

B. Roentgenogram of February 16, 1956, showing calcification of right hilar and right paratracheal areas and right apex; streaking extends upward from right root to apical calcification.

C. Bronchogram of August 29, 1956, showing narrowing of bronchus in anterior segment of the right upper lobe. On the original film a calcified node can be clearly seen just below this area of narrowing.

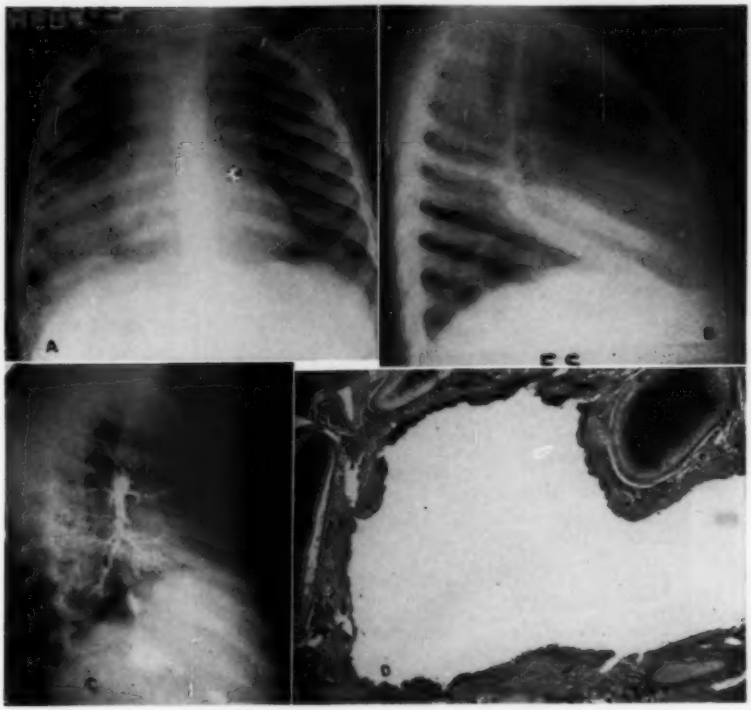

FIG. 5 (E.S.). *Bronchial stenosis.* Age, three years. Bronchoscopy of August 7, 1947, showed granulation tissue in right middle lobe orifice; duration to healing, four months. End result, stenosis of the right middle lobe bronchus. Streptomycin and PAS, three months. Resection of the right middle lobe on January 31, 1955.

A. Roentgenogram obtained on February 10, 1947, showing widening of the upper mediastinum to the right and an area of triangular clouding below the sixth posterior rib on the right.

B. Roentgenogram obtained on February 10, 1947, showing right lateral view; area of clouding corresponding to the right middle lobe.

C. Roentgenogram of August 13, 1953, indicating stenosis of right middle lobe bronchus.

D. Section of the right middle lobe showing a markedly ectatic bronchiole (× 25).

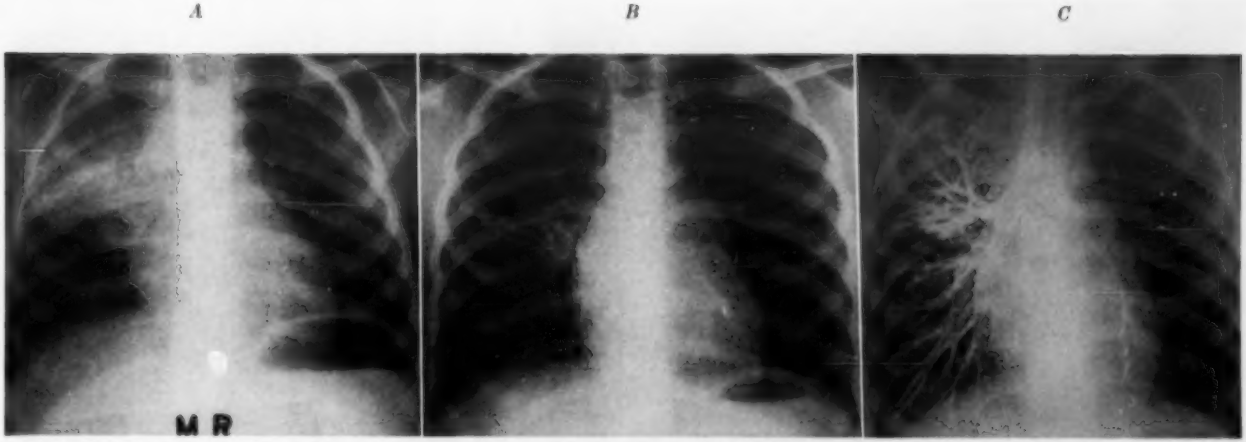

FIG. 6 (M.R.). *Cylindrical bronchiectasis.* Age, twenty months. Bronchoscopy of November 1, 1951, showed granulation tissue in the right main bronchus at the level of the right upper lobe orifice; duration to healing, ten months. Streptomycin and PAS, thirteen months.

A. Roentgenogram obtained on October 17, 1951, showing widening of upper mediastinum to the right and clouding in the second right interspace extending from root to axilla, containing a small area of increased radiolucency. On original roentgenogram, mottling is visible throughout this area and in the first right interspace.

B. Roentgenogram of March 26, 1956, showing elevated transverse fissure overlying the third right rib; small area of density may be noted just below fissure in midlung field; there are scattered calcifications in the right root area.

C. Bronchogram of September 5, 1956, showing cylindrical dilatation of bronchi in the anterior segment of the right upper lobe. Small area of calcification is visible in the second right interspace.

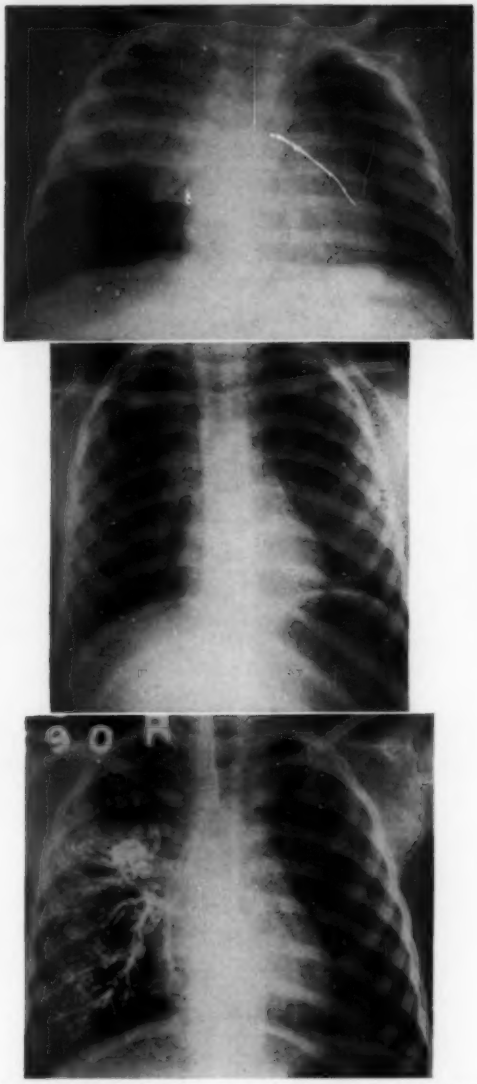

FIG. 7 (F.N.). *Saccular bronchiectasis.* Age, three months. Bronchoscopy of November 2, 1950, showed granulation tissue in the right upper lobe bronchus; duration to healing, seven weeks. Streptomycin three months, sulfone, one year.

A. Roentgenogram obtained on July 10, 1950, showing clouding of the right upper lung to lower border of the fourth anterior rib, more marked in lower portion of shadow.

B. Roentgenogram of June 17, 1953, showing enlarged right hilar shadow with heavy streaking extending outward into the first right interspace, also seen in the outer portion of the second interspace.

C. Bronchogram of July 23, 1953, showing saccular dilatation of bronchus to the anterior segment of the right upper lobe.

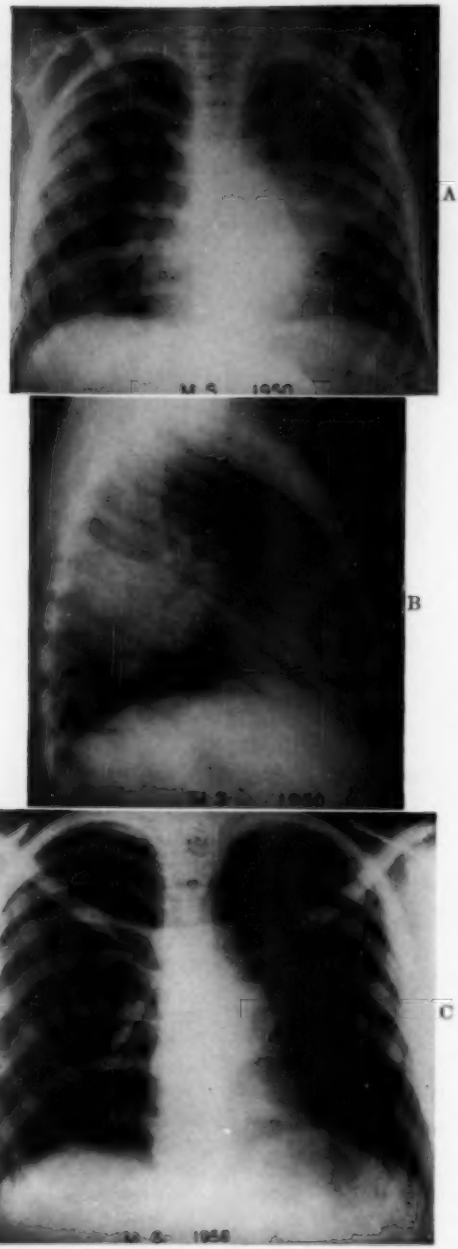

FIG. 8. (M.S.) Extensive calcification within segment originally obstructed; considered an indication for consideration for surgery. Age, 3 years. On May 15, 1950, the superior segmental bronchus of the left lower lobe was occluded with granulation tissue. The patient was treated for three weeks with corticotropin. Only very slight improvement occurred in fourteen weeks. The left lower lobe was resected on June 16, 1956.

A. Roentgenogram obtained July 7, 1950, showing triangular clouding of left lung adjacent to the hilum.

B. Lateral view, showing clouding in superior segment, left lower lobe (×6).

C. Roentgenogram obtained April 30, 1956, showing calcification in the left hilar area and through the heart shadow.

FIG. 8-*D-E*

D. Lateral view, showing extensive calcification in segment #6, left lower lobe.

E. Section of the left lower lobe showing residual caseum which has undergone partial granular calcification. (× 400)

SURGICAL THERAPY IN TUBERCULOSILICOSIS[1,2]

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(Received for publication May 16, 1957)

INTRODUCTION

It has long been known that the incidence of tuberculosis is high among patients with silicosis (1) and that the patient with concomitant tuberculosis and silicosis presents a more difficult problem in management than the patient with uncomplicated pulmonary tuberculosis. Dayman (2) has said that the average survival time after the development of symptoms or the appearance of acid-fast bacilli in the patient's sputum was two and a half years. Theodos and Gordon (3) state that approximately two-thirds of the patients with these two lesions will die within three years after the development of the manifestations of tuberculosis, although sanatorium care may prolong life in selected cases. A few patients with tuberculosilicosis have been treated surgically (2–6). In others, the conglomerate lesion of tuberculosilicosis has been considered to represent bronchogenic carcinoma, and only at surgery has the true diagnosis been established (7, 8).

MATERIALS AND METHODS

Forty-eight patients have been admitted to the Ohio Tuberculosis Hospital during the past six years with a history of occupational exposure to dust and with tuberculosilicosis either suspected or proved. Thirty of these patients have been treated by medical means, some because surgery was not indicated and others because pulmonary function or a cardiac condition precluded surgical therapy. Eighteen patients had surgical therapy. One patient was treated by extraperiosteal plombage, so tissue was not available for examination in this case. In 13 patients the surgical specimen revealed tuberculosilicosis, while in 4 nonspecific dust reaction was found in association with tuberculosis.

Since the criteria for the pathologic diagnosis of silicosis and of tuberculosilicosis are not generally familiar it is considered advisable to discuss and illustrate the methods by which the diagnosis was made in these patients. Histologic sections were prepared from the resected tissue in each of the 17 cases and were reviewed by one of the writers without knowledge of the occupational history or the roentgenographic appearance of the lesions. All of the specimens showed evidence of tuberculosis (figures 1 and 2). To establish the diagnosis of silicosis, particular attention was paid to the areas of tissue in which tuberculosis was absent or least conspicuous.

A case was not accepted as an example of tuberculosilicosis unless typical whorled nodules of hyalinized collagenous tissue could be found in areas distant from the tuberculous lesions (figure 3). Lesions showing central caseation were not considered to be silicotic nodules no matter how thick, whorled, and pigment-laden the capsule might be. Since organized tubercles can closely mimic silicotic nodules (figure 4), another requirement for diagnosis was the presence of excess pigmentation with fibrosis in the adventitia of

[1] From Ohio Tuberculosis Hospital, and the Division of Thoracic Surgery, Department of Surgery, Ohio State University Health Center, Columbus, Ohio.

[2] Presented before the Medical Session, as part of *Session 6*, at the annual meeting of the National Tuberculosis Association, Kansas City, Missouri, May 8, 1957.

FIG. 1. Photomicrograph of cavitary tuberculosilicosis. Note silicotic nodule projecting into lumen at right and paucity of inflammatory reaction over it. (120 ×)

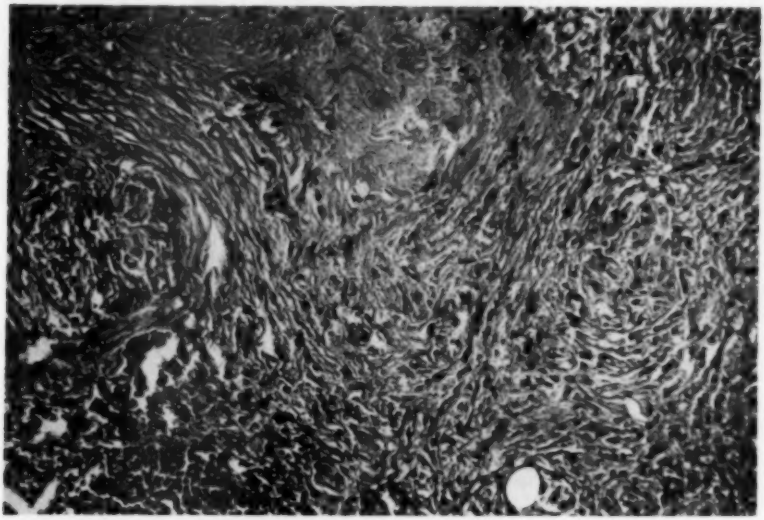

FIG. 2. Photomicrograph through an area of tuberculosilicosis. Silicotic nodule at upper center and tubercles at right and left, with intervening diffuse scarring. (120 ×)

FIG. 3. Typical whorled pigmented collagenous silicotic nodule. (120 ×).

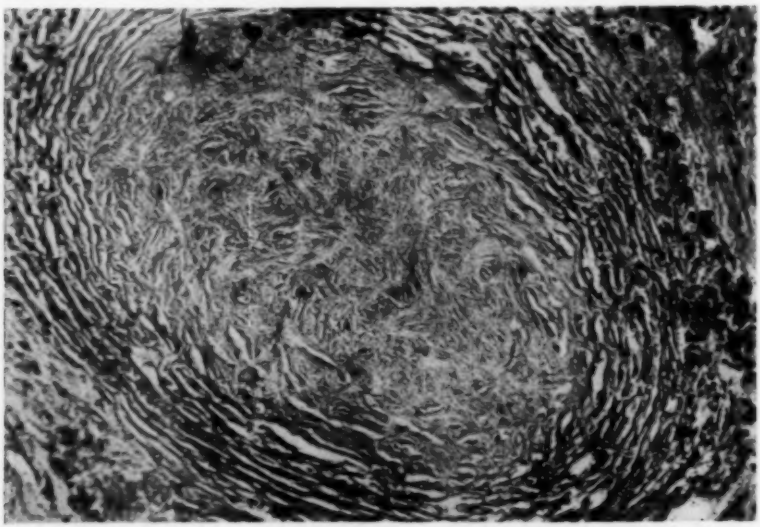

FIG. 4. Organized tubercle which mimics a silicotic nodule. (120 ×)

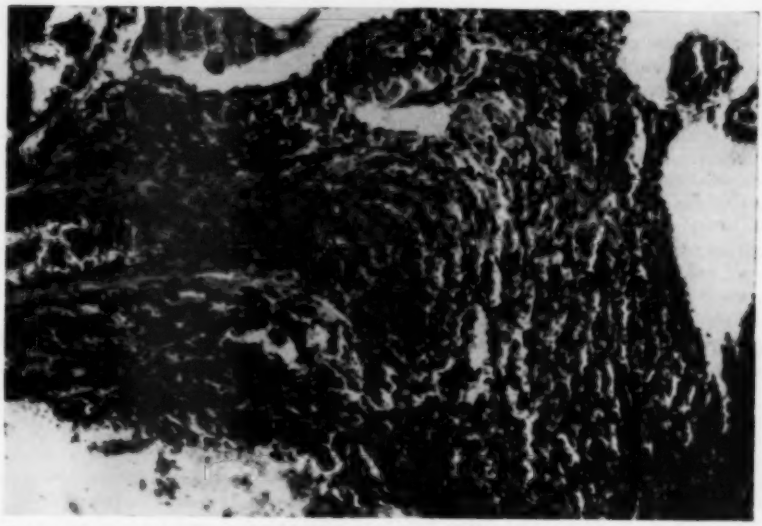

FIG. 5. Pigmentation and minimal fibrosis in adventitia of blood vessel. The degree of fibrosis here is the smallest amount considered adequate to permit diagnosis of silicosis in the presence of nodulation. (120 ×)

FIG. 6. Subpleural pigmentations and diffuse fibrosis. (120 ×)

bronchi and vessels, secondary lobular septa and pleura (figures 5 and 6). Thus, no case of tuberculosis was considered to be complicated by silicosis unless the tissue showed in noninfected areas both silicotic nodules and evidence of dust deposition and fibrosis in a lymphatic distribution.

These are the best criteria for the recognition of silicosis and of tuberculosilicosis. In the past it has been recommended that reliance be placed upon chemical analysis of tissue or demonstration of crystalline material by means of polarized light. Tissue chemistry is rarely helpful because the concentration of silica obtained often falls within the range of overlap found on analysis of a series of known silicotic and nonsilicotic lungs (9). Visualization of silica particles in balsam-mounted tissue sections is difficult because they are small and only slightly brighter than the background. Frequently they are obscured by the larger, brighter, and more numerous crystals which are probably fixation artifacts mistaken by many workers for quartz particles.

Of the 4 patients considered to have nonspecific dust reaction only one had a history of exposure to quartz-containing dust, as a chipper in a foundry for twelve years. None of the 4 patients showed convincing evidence of silicosis by roentgenography and none presented any of the technical difficulties observed in the patients with tuberculosilicosis. These 4 patients were eliminated from the study.

In the 14 patients with tuberculosilicosis the primary occupational exposure to silica dust was in potteries, foundries, and in work with firebrick. The period of active exposure ranged from three to forty years (table 1). The 2 patients with only three years' exposure had subsequently worked in nondusty areas for fourteen and twenty-five years.

A review of the drug therapy revealed that 12 patients had received chemotherapy for eight months or more prior to the surgical procedure and 2 for less than eight months. Thirteen patients received streptomycin and PAS, and one isoniazid and PAS. Drug-susceptibility studies demonstrated that 6 patients had tubercle bacilli susceptible to the chemotherapeutic agents received at the time of surgery. In 5 patients, drug-susceptibility studies could not be carried out because of the absence of cultures positive for tubercle bacilli, either from sputum or from the resected tissue. Three patients had organisms which were resistant to the available antituberculous chemotherapy and, in one of these, surgical collapse was performed in view of the known hazards of resectional surgery.

Sputum examinations for the purpose of this study were considered to be positive if tubercle bacilli were found by microscopy or culture during the three months prior to the operative procedure. By this criterion, 8 patients were considered to have infectious sputum during the preoperative period, and 6 to have noninfectious sputum.

The surgical procedures in the 14 patients with tuberculosilicosis were pneumonectomy in one, bi-lobectomy in one, lobectomy in 7, segmental resection or less in 4, and extraperiosteal plombage in one. Two patients had bilateral resections, and one had two operative procedures carried out on the same side, the first being a posterior segmental resection followed six months later by a right upper lobectomy when the disease progressed in this area.

Ten patients were considered to have silicosis in association with far advanced tuberculosis; 3, with moderately advanced tuberculosis; and one, with minimal tuberculosis.

TABLE 1

EXPOSURE TO SILICA

	Cases	Years
Pottery	3	10–40
Foundry	10	3–30
Firebrick worker	1	40

Results

The lesions found at surgery were open cavity in 11 patients, one of whom was treated by extraperiosteal plombage; filled cavity in one; and nodular disease in 5. Of the 16 resected specimens, cultures were positive for tubercle bacilli in 6, organisms were demonstrated by acid-fast stains of the tissue in 7 and, from one specimen, atypical acid-fast organisms were cultured. In one, acid-fast bacilli could not be demonstrated by any of these means, and in one additional patient these studies were not done.

Postoperatively 8 patients had sputum negative for tubercle bacilli. Five of these patients had been in the noninfectious category prior to surgery. Three patients had an isolated finding of tubercle bacilli in the sputum by microscopy or culture during the early postoperative course, and 3 had persistently infectious sputum. One of the latter was treated by extraperiosteal plombage and had tubercle bacilli in the sputum for nine months following the collapse procedure, when he became noninfectious and has remained in that category. The other 2 patients developed bronchopleural fistulas during the postoperative period and continued to have tubercle bacilli in the sputum associated with this complication.

One of the distinctive problems associated with resectional surgery in patients with tuberculosilicosis is the difficult dissection of the hilar structures because of the massive tissue reaction adjacent to silicotic lymph nodes (figure 7). Because of the proximity of these lymph nodes to the pulmonary artery and the superior

Fig. 7. Wall of pulmonary artery showing fusion with surrounding fibrosis and silicotic nodule at lower right. (120 ×)

and inferior pulmonary veins, considerable difficulty may be encountered in the isolation of these structures. In one of the early patients dissection resulted in tears of both the apicoposterior and lingular arteries during a left upper lobectomy. In a second patient, a tear occurred in the posterior segmental artery and extended into the main pulmonary artery during a lobectomy. The tear in the main pulmonary artery was repaired following intrapericardial occlusion of the vessel so that pneumonectomy was avoided. In 5 other patients the fibrotic lymph nodes resulted in difficult dissection at the hilus. Two of these required peripheral ligation of the vessels at their segmental distribution and one, in whom upper and middle lobectomies were done, required peripheral control of the bronchi as well. One patient in whom a pneumonectomy was being performed required intrapericardial ligation of the artery and veins. In 6 of the 13 patients treated by resection, no technical problem was encountered.

Complications occurring in these 14 patients (table 2) consisted of a persistent pleural space in one patient and a bronchopleural fistula with empyema in 3 others, associated in one with spread of the tuberculosis. One patient treated by posterior segmental resection of the right upper lobe developed progressive cavitary disease in this lobe. When a lobectomy was done six months later it was found that, due to an anomaly of the pulmonary artery, the total arterial supply to the lobe had been divided at the original procedure. In the patient

TABLE 2

COMPLICATIONS FOLLOWING SURGERY

16 Resections

Pleural space	1
Bronchopleural fistula with empyema	3*
Contralateral spread	1*
Progressive disease	1
Thrombophlebitis	1
Pericarditis	1
None	9

* Same patient.

TABLE 3

POSTOPERATIVE FOLLOW-UP OBSERVATIONS

Present Status

Years Post-operatively	Arrested		Still Hospitalized	Home and Convalescent	Relapse	Dead
	Working	Not Working				
4–5	2					
3–4	2		1		1*	1
2–3		3				
1–2	1					
0–1			2	1		

* Now arrested, not working, 5½ years postoperatively and one year since relapse.

whose segmental and main pulmonary arteries were torn, a self-limiting pericarditis occurred. Thrombophlebitis of the superficial saphenous vein occurred in one patient who had previously had a thrombophlebitis of the brachial vein following cardiac catheterization, assumed to have been controlled ten days prior to surgery.

The current status of the patients is detailed in table 3. Of the 14 patients, 8

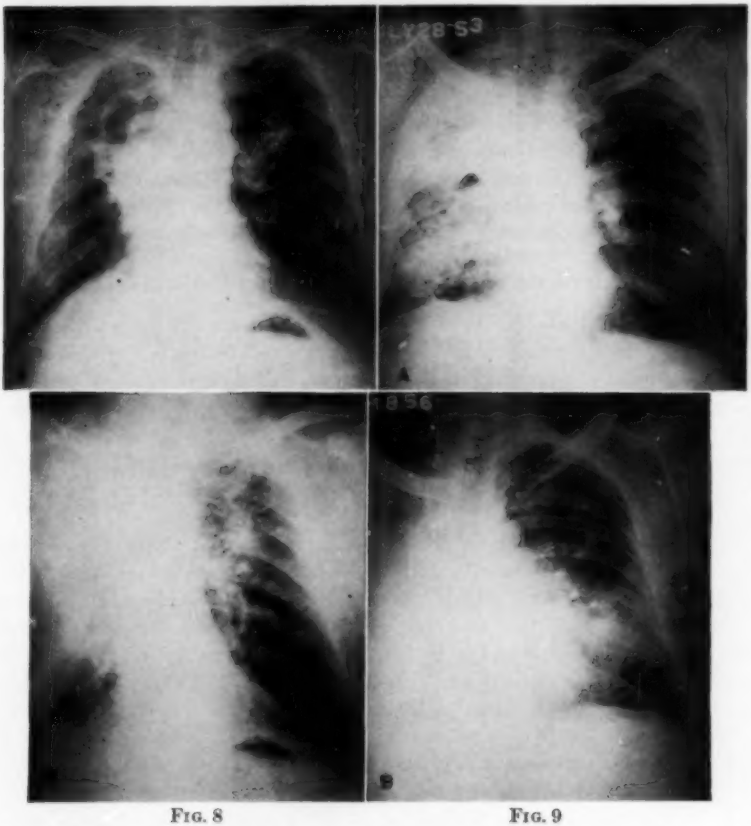

FIG. 8 FIG. 9

FIG. 8A. Far-advanced cavitary tuberculosilicosis, right, and conglomerate lesions, left. Note continuity of pulmonary lesion with hilus.

B. Following upper and middle lobectomies complicated by bronchopleural-cutaneous fistula. Patient still has fistula and infectious sputum despite thoracoplasty.

FIG. 9A. Far-advanced cavitary tuberculosilicosis. Note retraction of mediastinum, hilar fibrosis, and dense lesions throughout right lung.

B. Postoperative roentgenogram following pneumonectomy and thoracoplasty, showing complications of spread to contralateral lung and bronchopleural-cutaneous fistula. Patient died three years and five months following pneumonectomy.

are now considered to have arrested disease and 5 are working. Three patients, 2 of whom are still hospitalized and one of whom has recently been discharged, can be expected to return to an active, productive life. One patient who left the hospital against medical advice had tubercle bacilli in his sputum three years later. He was re-hospitalized and treated for twelve months and again left against advice. Despite this, the patient has had noninfectious sputum for more than one year. Two patients represent therapeutic failures. One who is still hospitalized developed a bronchopleural fistula during the early postoperative period which was not controlled with tailoring and several revision thoracoplasties. At the present time the patient continues to have infectious sputum three years following the removal of his right upper and middle lobes and a bronchopleural-cutaneous fistula is still present (figure 8). A second patient treated by a right pneumonectomy developed a bronchopleural-cutaneous fistula, not controlled by thoracoplasty, and a contralateral spread of tuberculosis (figure 9). Three years and five months after resection the patient died abruptly following a massive pulmonary hemorrhage.

Discussion

While few patients hospitalized with tuberculosilicosis will have a distribution of disease suitable for surgical intervention, some can be treated by this method. Careful selection and preoperative preparation, including ventilatory evaluation, is necessary for the successful outcome of surgical therapy, as it is in other patients in whom tuberculosis is associated with a second pulmonary lesion such as emphysema. The indication for surgery in these patients should be the presence of an open cavity or dense nodular disease associated with tubercle bacilli in the sputum.

Because there appears to be no satisfactory method of classifying associated tuberculosis and silicosis, the 14 patients with these combined lesions have been classified according to the extent of the pulmonary tuberculosis. It is assumed that the area of cavitation and conglomeration represents the primary area of tuberculous involvement and to this has been applied the standard classification for tuberculosis; namely, far advanced, moderately advanced, or minimal, disregarding the nodular infiltration throughout the remainder of the lung. While confusion might result in a patient with conglomerate lesions associated with both miliary tuberculosis and nodular silicosis, this was not present in any of the 14 patients observed. Utilizing this classification there appeared to be only a slight relationship between the extent of the pulmonary tuberculosis and the final outcome of the surgically treated patient. There was no relationship between the type of occupation or the period of exposure to silica and the final results. It appears that those patients who require the more extensive resectional procedures have an increased complication rate.

In the past, unilateral conglomerate lesions have been confused with bronchogenic carcinoma and have led to exploratory thoracotomy for diagnosis (7, 8). Careful preoperative studies, including sputum examinations, detailed history of occupational exposure, and the use of bronchoscopy and bronchograms should help differentiate between bronchogenic carcinoma and conglomerate tuberculo-

silicosis. A bronchogram will in most instances fail to show a bronchial block (10).

Kergin (8), in discussing 6 patients with silicosis and tuberculosilicosis in whom resections were accomplished, commented upon the difficulties associated with the surgical treatment of these lesions. In 2 of his patients in whom silicosis was not complicated with tuberculosis the pulmonary vein was torn in one and the pulmonary artery in a second. In the present group technical difficulties were encountered in 7 of the 13 patients who had had resection. Tear of the pulmonary artery has not required more extensive resection than was planned. Intrapericardial control of the pulmonary vascular system can be accomplished but may increase the postoperative morbidity because of the possibility of producing pericarditis. Peripheral control of the pulmonary vessels was a useful maneuver when the dense fibrotic lymph nodes were so situated that segmental or lobar isolation of these structures was impossible. However, the associated peripheral control of segmental bronchi during lobectomy represented an extreme hazard from the standpoint of the development of bronchopleural fistula. In many patients, however, despite the dense lymph nodes surrounding the hilar structures, careful dissection will allow the usual control of bronchi and vessels.

No conclusions can be drawn from the single patient in whom extraperiosteal plombage has been used successfully. Conflicting reports are present in the literature concerning collapse in the treatment of tuberculosilicosis. Maier and Hurst (11) reported an extrapleural pneumothorax which was successful in an individual with tuberculosilicosis, while Auerbach and Stemmerman (4) treated 4 patients with thoracoplasty, 2 of whom died following the first stage of thoracoplasty and 2 others following the third stage and revision thoracoplasty. All 4 had patent cavities. Dayman (2) reports one patient treated by thoracoplasty without improvement of his disease. Theodos and Gordon (3) state that cavity closure was seldom obtained by either thoracoplasty or artificial pneumothorax except in cases with early nodular silicosis. They do state that one patient treated by thoracoplasty had cavity closure and reversal of infectiousness.

In 2 of the 14 patients in the present study the treatment must be considered to have been a failure. Both had extensive resections for tuberculosilicosis and both developed bronchopleural fistulas following difficult intrapleural dissections. One lived for three years and five months with active disease in his remaining lung and later died from a massive pulmonary hemorrhage. The other has sputum positive for tubercle bacilli in association with a bronchopleural-cutaneous fistula.

Summary

Surgical therapy can be applied successfully to some patients with active tuberculosilicosis. Those patients with extensive disease, which requires a pneumonectomy or bi-lobectomy, in whom the roentgenogram demonstrates extensive lymph node involvement near the hilus, can be expected to have poor results. Technical difficulties will be encountered in many patients with tuberculosilicosis; however, by careful surgical dissection, by peripheral and intrapericardial control of the pulmonary artery and pulmonary veins, these difficulties can be surmounted. Of the several complications that may occur in these patients, bronchopleural fistula is the most hazardous and the most difficult to control.

With a careful combination of medical and surgical treatment a great proportion of the patients can be expected to return to active and useful lives.

Sumario

La Terapéutica Quirúrgica en la Tuberculosilicosis

A algunos enfermos que tienen tuberculosilicosis puede aplicárseles con éxito la terapéutica quirúrgica. Cabe esperar malos resultados en los sujetos con enfermedad extensa que requerirá una neumonectomía o bilobulectomía, en quienes la radiografía revela invasión difusa de los ganglios linfáticos cerca del hilio. En muchos enfermos de tuberculosilicosis, se encontrarán dificultades técnicas; sin embargo, con la cuidadosa disección quirúrgica y el control periférico e intrapericardíaco de la arteria pulmonar y las venas pulmonares, pueden sobrepujarse tales dificultades. De las varias complicaciones que pueden ocurrir en estos enfermos, la fístula broncopleural es la más peligrosa y la más difícil de dominar.

Después de una cuidadosa combinación del tratamiento médico y quirúrgico, cabe esperar que una gran proporción de los enfermos vuelva a llevar una vida activa y útil.

Resume

Traitement chirurgical dans la silico-tuberculose

Le traitement chirurgical peut s'appliquer avec succès à certains cas de silico-tuberculose en activité. Chez les malades atteints de lésions extensives justiciables de la pneumonectomie ou de la double lobectomie, chez lesquels les radiogrammes révèlent une adénopathie hilaire extensive, il faut s'attendre à de mauvais résultats. Des difficultés techniques surgiront dans de nombreux cas de silico-tuberculose. Néanmoins, la dissection chirurgicale minutieuse et le contrôle périphérique et intrapéricardique de l'artère pulmonaire et des veines pulmonaires permettront de surmonter ces difficultés. Parmi les diverses complications susceptibles de se produire chez ces malades, la fistule bronchopleurale est la plus dangereuse et la plus difficile à contrôler.

Lorsque le traitement médical et chirurgical sont soigneusement combinés, une grande proportion des malades est appelée à reprendre une vie active et utile.

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PNEUMONECTOMY IN PULMONARY TUBERCULOSIS[1]

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(Received for publication June 18, 1957)

INTRODUCTION

There were many significant events in the development of thoracic surgery which finally led to the first successful pneumonectomy, all of which are now only of historic interest.

In 1930, E. D. Churchill, after finding it necessary to use individual ligation of the hilar structures because of the proximity of tumor, failed to have a successful pneumonectomy only because of Sauerbruck's warning about mediastinal emphysema following bronchial closure. Because of this influence he placed a tube from the bronchus to the exterior, and the patient died three days later of bronchial pneumonia. In 1931, Rudolf Nissen (1), formerly of Berlin, using a mass ligature of the hilum and allowing the lung to slough out, removed the lung successfully in a twelve-year-old girl. The second successful pneumonectomy was performed in 1932 by Cameron Haight of Ann Arbor, Michigan, for bronchiectasis; he used the same technique. The third successful operation represented a twofold advance and was performed in April, 1933, by the late E. A. Graham. It was carried out in one stage, using Harold Brunn's technique, the weak point of which lay in the stump closure. A breakdown of the bronchial closure made revision thoracoplasty necessary a few weeks later.

Rienhoff made valuable observations relative to management of the pulmonary hilum by individual ligation technique, and no true essential changes in technique have since taken place. In 1933 he did a pneumonectomy on a three-year-old child with fibrosarcoma. He isolated and ligated the three vessels and transected the bronchus, closing this with interrupted sutures, re-enforcing with a flap of pleura.

For the next several years the mortality from the procedure remained high. No antimicrobial drugs were available and few blood transfusions were employed. It was not until the development of penicillin and the antituberculous drugs that excision of lung tissue for tuberculosis became an acceptable procedure. Although the late John Alexander's influence did much to stimulate surgeons throughout the world to employ surgical therapy for pulmonary tuberculosis, it is interesting to note a paragraph from his second book which appeared in 1937.

> In view of the excellent clinical results that may be obtained from combination of the sanatorium regimen and collapse therapy, the removal of a portion of lung can no longer be considered for early or limited lesions. The resection of a portion of a tuberculous lung is inevitably a hazardous operation, whereas the collapse therapy operations that are used for limited lesions are attended by very little danger.

In 1943, Churchill and Klopstock revived interest in the possibilities of lobectomy for tuberculosis, but Maier and Sweet (2) concluded that the end results from a three-year follow-up study were not satisfactory. Indeed, until 1942, only 75 resections for pulmonary tuberculosis had been reported, Freedlander (3) having performed the first successful, planned resection for pulmonary tuberculosis in 1934; he reported this in 1935.

[1] From Battey State Hospital, Rome, Georgia.

Gale, Dickie, Rankin, and Curreri (4) reported at the meeting of the National Tuberculosis Association in 1955 on a total of 1,165 patients who had had pulmonary resection from the year 1944 to 1954. There were 252 pneumonectomies with a mortality of 8.3 per cent, the most common causes of death being bronchopleural fistula, tuberculous pneumonia, and respiratory failure.

Hirdes and Bosch (5) of Holland reported 600 pulmonary resections with 106 pneumonectomies from October, 1949, to October, 1953. They reported a case mortality of 6 per cent, and only 7 of the patients continued to discharge tubercle bacilli postoperatively. Among 99 patients, 3 were found to have a spread of disease and 3 experienced reactivation of disease. Based on their statistical data, Hirdes and Bosch recognized no need for a thoracoplasty in the pneumonectomized patient.

Bjork (6) of Sweden reported 151 pneumonectomies in 1956, with a mortality of 10.6 per cent. There was a cure rate of 87 per cent. He found that 65 per cent of the cured patients were working full time and 11 per cent were doing a half day's work.

Robinson, Jones, Myers, and Redding (7) reported in May, 1955, the results of surgery for tuberculosis in 1,363 patients. Eighty per cent of these were private patients and 20 per cent had been cared for in two tax-supported hospitals. There were 141 pneumonectomies, with an early and late mortality of 14 per cent. They showed a mortality of 19 per cent when the procedure was performed on the right side, as compared with 10.3 per cent on the left side.

Overholt, Wilson, and Gehrig (8) reported in 1952 on 152 pneumonectomies in the pre-streptomycin era. Thirty-one per cent of these patients had tuberculous complications, and there was a mortality of 15 per cent. This group could be compared with 137 pneumonectomies during the streptomycin era with 6.5 per cent of tuberculous complications and a mortality of 5.8 per cent.

A cooperative study from the Veterans Administration (9), covering 3,657 pulmonary resections over the period from July, 1952 to July, 1955, showed 185 pneumonectomies with a 16.5 per cent mortality and an 18.5 per cent incidence of empyema. A subsequent report on 1,388 resections for pulmonary tuberculosis, with 74 pneumonectomies from July, 1955, to July, 1956, showed a surgical mortality of 8 per cent. Fourteen per cent of these patients developed fistulas and 15 per cent had tuberculous empyema. This represented some improvement over previous reports.

Materials and Methods

The material in the present paper represents a study of the pneumonectomies performed at Battey State Hospital from 1948 to 1957. During this period of time 746 pulmonary resections were carried out, 119 (16 per cent) of these being pneumonectomies.

Although the pneumonectomies in this study have been performed by surgeons who had training in three separate medical centers, the actual technique and postoperative care has varied but little.

Almost all of these patients were operated upon in a face-down position. The chest was entered through the fifth intercostal space, after short paravertebral segments of the fifth and sixth ribs were removed. The pulmonary artery and both veins were doubly ligated with large cotton. The bronchus was closed with interrupted sutures of fine cotton and was usually transected high enough to allow it to retract behind the mediastinal pleura for coverage; otherwise, a flap of parietal pleura was sutured over the stump for additional coverage. The pneumonectomy space was irrigated with 3,000 ml. of saline and, if the space

TABLE 1

PNEUMONECTOMIES BY YEAR

1948	2
1949	13
1950	7
1951	8
1952	14
1953	14
1954	20
1955	21
1956	20

TABLE 2

AGE AND RACE DISTRIBUTION

Age	White		Negro	
	M	F	M	F
12–19	1	2	3	3
20–29	2	9	10	16
30–39	7	5	5	16
40–49	5	7	8	8
50–59	4	5	2	0
60–64	0	1	0	0

was thought to have been contaminated, 6,000 ml. of saline were used. A No. 26 fenestrated catheter was placed in the pneumonectomy space and connected to underwater seal for drainage and usually left in for forty-eight hours. This was done regardless of the absence or presence of a thoracoplasty. It is believed that this is quite safe and simplifies the postoperative care, since pressure readings are not necessary. There has been no incidence of overshifting of the mediastinum to the operated side, and mediastinal swing has been of no great concern. In 2 patients who continued to bleed, a severe shift to the nonoperated side occurred, since the thoractomy tube had ceased to drain because of clotting. The amount of drainage from the chest has served as an indication of the need that blood be given in the postoperative period. The blood loss during surgery was measured and replaced accordingly.

A concomitant six-rib thoracoplasty (two through seven) was usually done if the patient was in good condition and if blood loss had not been excessive. In a recent comparative study by Shek and Myers (10) at this institution, it was shown that this combined procedure did not add to the morbidity or mortality in the 34 consecutive cases studied.

In table 1 may be seen the distribution of pneumonectomies by year. Approximately two-thirds of these pneumonectomies have been carried out after the advent of isoniazid therapy. In table 2 may be seen the age and race distribution, the youngest patient being a Negro female, age twelve years, and the oldest a white female, age sixty-four.

OBSERVATIONS

The present series of patients represents chiefly a group of so-called "salvage" cases, with an average duration of hospital treatment of thirty-eight months before surgery (table 3). Ninety-seven of these patients were described as having "destroyed" lungs. There were 14 definitive thoracoplasty failures; 5 plombage

failures; 2 patients had torn pulmonary arteries, necessitating pneumonectomy; and one patient was thought to have a malignancy. The classification on admission showed 116 patients to have far advanced pulmonary tuberculosis, and 3 patients were diagnosed as having moderately advanced disease.

The bacteriologic status of the sputum at the time of resection may be seen in table 4. One patient in the infectious group was found to have atypical microorganisms, as determined by culture and guinea pig inoculation. There had been no preoperative drug therapy in this case which was treated in the early days of chemotherapy. Streptomycin and PAS were given for eight months postoperatively, however, and the patient had a benign course and has since remained well and able to work.

The tuberculous complications in the group of patients with tubercle bacilli in the sputum may be seen in table 5. Thirty-nine per cent of these patients had tuberculous complications. Thirty-seven per cent of the patients with drug-resistant strains of tubercle bacilli and 43 per cent of the patients with drug-susceptible strains had tuberculous complications. In this "drug-susceptible" group, the *in vitro* tests, in many instances, had not been done for as long as six months prior to the date of surgery. It is believed that this explains, in some measure, the high percentage of complications in the "drug-susceptible" group. Many may actually belong in the "drug-resistant" group.

Only 3 of the 7 patients with bronchopleural fistulas are at home and well. Two patients died from this complication and 2 remain in the hospital, each with a tube in a small sinus tract. Both have had thoracoplasties. Only one of the 7 patients who developed bronchopleural fistulas was not treated with tube drainage. She had a thoracoplasty on the nineteenth postoperative day after a bronchopleural fistula had been proved with the instillation of Lipiodol® in the trachea. At surgery, the pleura was torn and she developed a bronchopleurocutaneous fistula which drained for one year. She is now at home and well.

TABLE 3

SURGICAL INDICATIONS

Destroyed lung	97
Thoracoplasty failure	14
Plombage failure	5
Torn pulmonary artery	2
Missed diagnosis (thought to be carcinoma)	1

TABLE 4

SPUTUM STATUS AT RESECTION

	"Drug-resistant"	"Drug-susceptible"	Total
Positive for tubercle bacilli	62	23	85
Negative for tubercle bacilli	0	0	34

TABLE 5

TUBERCULOUS COMPLICATIONS IN GROUP WITH INFECTIOUS SPUTUM

	"Drug-resistant" Strains	"Drug-susceptible" Strains	Total
Bronchopleural fistula	5	2	7
Tuberculous empyema	5	3	8
Tuberculosis of chest wall	2	0	2
Spread	4	0	4
Disease of bronchial stump	12	7	19
Total	28 in 23 patients	12 in 10 patients	40

TABLE 6

NONTUBERCULOUS COMPLICATIONS

Tachycardia	4
Staphylococcal infection, chest wall	1
Pulmonary edema	2
Postoperative hemorrhage	6
High fever	6
Dyspnea—severe	3
Psychosis	2
Cardiac arrest	1
Total	25

There were no tuberculous complications found in the group of 34 patients whose sputum was negative for tubercle bacilli at the time of surgery.

The incidence of nontuberculous complications was approximately evenly divided between the infectious and noninfectious patients (table 6). Eighty-one patients had essentially benign postoperative courses. Eight patients either died at surgery or were dead within five days thereafter. In the remaining 30 patients there was a total of 65 complications, 40 of which were tuberculous and occurred in the group with tubercle bacilli in the sputum at the time of surgery.

Two of the patients who bled postoperatively were carried back to the operating room in critical condition, with a severe shift of the mediastinum to the nonoperated side. A large hematoma was removed from the pneumonectomy space without the isolation of any significant bleeding point. Both of these patients survived after a total of thirty and thirty-two 500 ml. transfusions of blood, respectively, had been given. The 6 patients with high fever were thought to have probably had empyemas which subsided under drug therapy. Cultures of pleural fluid were not taken. There were 3 patients with severe dyspnea that extended beyond their immediate postoperative period. At the time of discharge from the hospital, however, they were no longer complaining of shortness of breath. Of the 2 psychotic patients, one is in a mental institution and the other recovered after being sent home.

TABLE 7

SURGICAL DEATHS WITHIN SIXTY DAYS

	"Drug-resistant" Strain	"Drug-susceptible" Strain	Total	Per Cent
Patients with tubercle bacilli in sputum	6	2	8	9.4
Patients without tubercle bacilli in sputum	0	0	2	6.0
Total			10	8.4

In one patient, cardiac arrest occurred after she was turned over on the operating table. The arrest was recognized immediately and cardiac massage was begun within three or four minutes. After five minutes no return of action was observed and 5 ml. of 5 per cent calcium chloride were then instilled into the left ventricle, following which there was immediate return of a normal heart beat. The patient was in coma for about 20 hours, following which she began to respond, and over the next three to four weeks showed signs of cerebral damage. On being discharged home six months later, she was completely free of any symptoms. This patient has since remained well and is now at work.

The mortality rate in the infectious sputum cases was 8 of 85 (9.4 per cent), but the death rate was three times greater in the "drug-resistant" group than in the "drug-susceptible" group (table 7). One of the 2 deaths in the noninfectious group occurred from cardiac arrest on the table, and the other patient died five hours postoperatively from pulmonary insufficiency. The 8.4 per cent represents the total surgical mortality.

Survey of Surgical Deaths (Table 8)

Case Reports

Case 1. The patient G.H. was a Negro female, age 12½ years, with stenosis of the right main stem bronchus and with tubercle bacilli in the sputum. She had a right pneumonectomy on August 29, 1950. There was a blood loss of 1,460 ml., and she was thought to have been in fair condition in her immediate postoperative period. Three hours before death she became quite restless and was given 0.005 mg. of morphine sulfate. She died thirty-seven hours after the operation; there was no autopsy and the exact cause of death was undetermined.

Case 2. The patient G.A.W. was a Negro male, age 31 years, with tubercle bacilli in the sputum. He had severe dyspnea following surgery and died on the fourth postoperative day with pulmonary insufficiency. He was thought to have developed acute pulmonary edema and was bringing up pinkish-white, frothy sputum. Chest roentgenograms showed fluffy, soft shadows consistent with that of pulmonary edema throughout the remaining lung.

Case 3. The patient C.C.P. was a white female, age 50 years, who was critically ill on admission three years previously. She had been bedridden and unable to walk up to one year before surgery. She had extensive saccular and tubular bronchiectasis throughout

TABLE 8

SURVEY OF SURGICAL DEATHS

Cause undetermined	1
Pulmonary insufficiency	2
Pulmonary edema	2
Cardiac arrest	3
Reopening of bronchial stump	2

the right lung, with a huge system of cavities. Her sputum had last contained tubercle bacilli thirty-one months before surgery. The specimen of lung removed was negative for tubercle bacilli on microscopy and culture, and her cavities were classified as healed. She had a right pneumonectomy on February 21, 1956, with a blood loss of 5,000 ml. She died five hours after the operation with pulmonary insufficiency. An autopsy showed a moderate degree of fibrosis of the lung with a healed, small tuberculous cavity and a small amount of atelectasis in the left upper lobe.

Case 4. The patient M.K. was a white female, age 37 years, with infectious sputum. She represented a plombage-thoracoplasty failure. A left pneumonectomy was carried out on November 7, 1951. There was a measured blood loss of 3,500 ml. and she bled postoperatively, 800 ml. being measured during the first three hours and 2,000 ml. during the next eight hours. She died suddenly on the fifth postoperative day. An autopsy showed acute congestion of all organs and a partially obstructing hemorrhagic mucous plug of the trachea and the right main stem bronchus. There were also interstitial and interparenchymatous pulmonary hemorrhages throughout the remaining lung. The patient was thought to have died from acute pulmonary edema.

Case 5. The patient R.L. was a Negro male, age 41 years, with infectious sputum. He represented a thoracoplasty failure, having had seven ribs removed two years previously. He had a right pneumonectomy on October 20, 1952. There was a blood loss of 600 ml., and the patient did well for twenty-four hours. He then developed sudden respiratory difficulty with clinical evidence of acute pulmonary edema, and died suddenly. An autopsy showed right ventricular hypertrophy and some dilatation to twice the normal size and thickness. This patient was a diabetic. He was not thought to have had excessive fluids given intravenously, having had only 1,000 ml. of 10 per cent glucose in water with insulin and 2,000 ml. of blood during and after the surgery.

Case 6. The patient M.L.S. was a Negro female, age 31 years, with sputum that had been negative for tubercle bacilli for six months before surgery. A left pneumonectomy was done on February 17, 1953, with completion of an eight-rib thoracoplasty, a three-rib preresection thoracoplasty having been performed previously. The blood loss was 1,450 ml. This patient had a sudden cardiac arrest as the chest was being closed, and cardiac massage with resuscitation measures failed. The left pulmonary artery had been found to be thrombosed at the time of surgery. This patient was never cyanotic and she had had satisfactory cardiac rhythm and blood pressure throughout the procedure. Autopsy findings were essentially negative except for moderate dilatation of the right ventricle. It was believed that her cardiac arrest had most likely been precipitated by a right ventricular failure. Cultures and smears from the resected lung were negative for acid-fast bacilli.

Case 7. The patient I.R.W. was a white female, 56 years old, with infectious sputum and a destroyed lung on the left side. A left pneumonectomy was done on June 16, 1949, and there was a blood loss of 2,000 ml. The patient had a cardiac arrest while the hilar dissection was being carried out. A tension pneumothorax on the contralateral side was

recognized at once and was relieved by placement of a tube. She responded well to cardiac massage, and the cardiac action returned to what was considered a normal state. The pneumonectomy was then completed, but the patient died six hours later, apparently from brain damage which occurred at the time of the cardiac arrest. This patient, incidently, had *situs inversus*.

Case 8. The patient B.B. was a Negro male, age 44 years, with infectious sputum. He had a left pneumonectomy on July 20, 1956, and did poorly throughout the operation. There was a moderate degree of cyanosis and tachycardia which could not be relieved. The patient had a blood loss of 1,700 ml. His blood pressure was normal until about thirty minutes before the chest was closed, when it dropped to shock levels. Cardiac arrest occurred at the time the chest was closed, and cardiac massage was begun within about two minutes. Resuscitation measures failed; no autopsy was obtained. This patient was considered a good surgical risk and the cause of his hypoxia was never determined.

Case 9. The patient M.S. was a Negro male, age 43 years, with tubercle bacilli in the sputum that were resistant to all drugs. He had a destroyed right lung and had had a five-rib thoracoplasty on the right side as an emergency measure to control hemoptysis some six months previously. He was thought to have shown improvement. There had been no further hemoptysis, and subsequently a right pneumonectomy was done with completion of a seven-rib thoracoplasty. Grossly, as well as microscopically, the patient was found to have tuberculous endobronchitis, extending into the margin of resection. He did well until the twenty-ninth postoperative day when he developed a bronchopleural fistula, along with a tuberculous empyema. This was relieved by having a tube placed in his chest, but apparently drainage was not adequate, and he died suddenly on the forty-first postoperative day. Autopsy showed a soiled left tracheobronchial tree from aspiration from the empyema space.

Case 10. The patient J.L. was a Negro male, 26 years old, with drug-resistant tubercle bacilli in the sputum. A right pneumonectomy and concomitant seven-rib thoracoplasty were carried out on August 17, 1955. There was a blood loss of 3,600 ml. The patient had tuberculous endobronchitis, but this did not extend into the margin of resection. There also was a destroyed lung. He bled postoperatively and was in shock intermittently for twenty-four hours. Ten blood transfusions of 500 ml. each were given during the evening and the night following surgery. He then did well until the thirty-sixth postoperative day when the ligatured bronchial stump reopened. A tube was placed in his chest, but apparently drainage was inadequate. The patient died five hours later and no autopsy was obtained. Fluid removed from his pneumonectomy space was positive for tubercle bacilli.

Postoperative Sputum Status

In the 34 patients with noninfectious sputum at the time of surgery, there were two deaths, one from cardiac arrest and another from pulmonary insufficiency. The remaining 32 patients have since remained in the noninfectious category. In the group of 85 patients with infectious sputum at the time of surgery there were eight surgical deaths. Of the remaining 77 patients, 33 (40 per cent) had tubercle bacilli in the sputum at some time during their postoperative period and 44 (60 per cent) consistently had noninfectious sputum postoperatively. Twenty-three of these patients whose sputum contained tubercle bacilli postoperatively had drug-resistant strains and 10 had drug-susceptible strains. In accounting for the source of the tubercle bacilli in the "drug-susceptible" group of patients, it was found that there were 7 patients with proved disease of the bronchial stump and 2 with bronchopleural fistulas. The source of the infectious

TABLE 9
MORTALITY

	Number	Per Cent
Surgical	10	8.4
Late (tuberculosis 1; nontuberculous 4)	5	4.2
Total	15	12.6

sputum in one case remained unexplained. In the 23 patients with drug-resistant strains of tubercle bacilli postoperatively there were 12 with proved disease of the bronchial stump; 2 suspected of having this complication; 4 with proved spread of the pulmonary tuberculosis; and 5 with bronchopleural fistulas. All of these patients in both the "drug-susceptible" and "drug-resistant" groups, who had proved disease of the bronchial stump, were treated locally with silver nitrate and aerosol instillations and removal of stump sutures when found. This has proved successful in eliminating the discharge of tubercle bacilli in the sputum.

Follow-up Observations

The over-all surgical and nonsurgical mortality in the series was 12.6 per cent (table 9). The one late death from tuberculosis was due to tuberculous pericarditis six months following surgery in a patient discharging tubercle bacilli which were completely resistant to the major antituberculous drugs. Of the 4 deaths from nontuberculous causes, 2 patients died in automotive crashes, and 2 died of cor pulmonale. Of the 104 living patients, 103 are known to have noninfectious sputum. One patient with infectious sputum and cavitary disease is at home. Two of the 7 patients who had bronchopleural fistulas remain in the hospital with tuberculous sinus tracts from their empyema space, but with noninfectious sputum. These 101 patients remain well and show a total "salvage" rate of 85 per cent.

SUMMARY

A brief review of the literature on pneumonectomy in the treatment of pulmonary tuberculosis has been made and the cases of 119 patients who had pneumonectomy at Battey State Hospital, Rome, Georgia, from 1948 to 1957 have likewise been reviewed.

The tuberculous complications in the patient with noninfectious sputum are nil, but remain high in the patient with infectious sputum. As expected, the nontuberculous complications are evenly divided between both groups. The reported evidence of bronchopleural fistula in 6.3 per cent and surgical mortality of 8.4 per cent compares favorably with evidence reported in other series. The death rate was three times greater in the "drug-resistant" than in the "drug-susceptible" patient. The mortality remains high from this procedure and did not decrease during the nine years represented by the present series.

Sumario

La Neumonectomía en la Tuberculosis Pulmonar

Ofrécese una breve reseña de la literatura relativa a la neumonectomía en el tratamiento de la tuberculosis pulmonar. Repásanse los casos de 119 enfermos a quienes se ejecutaron neumonectomías de 1943 a 1957 en el Hospital Estatal Battey de Rome, Georgia.

Las complicaciones tuberculosas en un enfermo cuyo esputo no sea infeccioso son nulas, pero continúan siendo altas en el enfermo de esputo infeccioso. Como se esperaba, las complicaciones no tuberculosas se dividen igualmente entre los enfermos de esputo positivo o negativo para bacilos tuberculosos. Los datos comunicados de fístula broncopleural en 6.3 por ciento y la mortalidad quirúrgica de 8.4 por ciento se comparan favorablemente con los datos presentados en otras series. La tasa de mortalidad fué tres veces mayor en los enfermos farmacorresistentes que en los farmacosusceptibles. La mortalidad permaneció alta y no disminuyó.

Resume

Pneumonectomie dans la tuberculose pulmonaire

Une revue sommaire de la bibliographie sur la pneumonectomie dans le traitement de la tuberculose pulmonaire est exposée. Les auteurs analysent 119 observations de malades soumis à la pneumonectomie au Battey State Hospital, Rome, Georgia, (U.S.A.) de 1948 à 1957.

Les complications de la tuberculose chez le malade dont l'expectoration est dépourvue de bacilles sont nulles, mais elles demeurent élevées chez le malade bacillifère. De toute évidence, les complications non tuberculeuses se répartissent à égalité entre les deux groupes. Les résultats consistant en fistules broncho-pleurales dans 6,3% des cas et une mortalité chirurgicale de 8,4% ne le cèdent en rien à ceux rapportés dans d'autres séries. Le taux de mortalité est trois fois plus élevé dans les cas résistants que dans les cas sensibles à la chimiothérapie. Le taux de mortalité est demeuré élevé.

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PYRAZINAMIDE AND VIOMYCIN IN THE SURGICAL TREATMENT OF PULMONARY TUBERCULOSIS[1, 2]

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(Received for publication April 25, 1957)

Introduction

In recent years the proportion of tuberculous patients with tubercle bacilli resistant to streptomycin, PAS, and/or isoniazid considered for pulmonary surgery is increasing. Many of these people have been ill for a number of years and have received either intermittent or single-drug therapy in the past, when such regimens were popular. Others have been too ill for surgery and have received antituberculous drug therapy in the hope that they would improve sufficiently to permit surgery at a later date. Many patients for numerous reasons have not remained long enough in any institution to receive adequate therapy, although it is unusual to encounter in this group a patient who has not received considerable amounts of streptomycin, PAS, and isoniazid. Relatively few of these patients, however, have had the benefit of continuous long-term antituberculous drug therapy from the time of their initial chemotherapy.

When planning pulmonary resection for a patient with organisms resistant to streptomycin, PAS, and isoniazid, the present writer at first merely added pyrazinamide (usually 1 gm. three times a day) to the existing drug regimen. Viomycin (2 gm. twice a week) was used cautiously at first because of the early reports by Walkup and Murphy (1) of its toxicity and poor results. Since this writer is convinced that multiple-drug therapy is superior to single-drug therapy, pyrazinamide and viomycin were given together as soon as it became evident that they would be well tolerated. However, whenever the patient's tubercle bacilli were susceptible to other drugs, namely, streptomycin, PAS, or isoniazid, these, were employed as part of the multiple-drug therapy. Occasionally cycloserine, oxytetracycline (Terramycin®), thiosemicarbazone (Tibione®), and/or a sulfone (Promizole®) have been used, but only when other drugs have not been available.

Materials and Methods

From May 1, 1954, to November 1, 1956, 54 patients received pyrazinamide and/or viomycin in the treatment of surgical conditions at the Ray Brook State Tuberculosis Hospital. The indications are summarized in table 1 and reviewed in detail in tables 2 to 5.

In this study only "effective" drug therapy is listed, that is, only those drugs to which the patient's tubercle bacilli were susceptible at the time of surgery. Except in the early cases in this series, drugs to which the patient's tubercle bacilli were resistant were discontinued prior to surgery. Drug susceptibility was determined by inoculating the patient's tubercle bacilli upon egg medium (Hohn's) containing 10 γ of streptomycin, 10 γ of PAS, and 5 γ of isoniazid. Growth in the presence of a drug was considered evidence of resistance

[1] From the Ray Brook State Tuberculosis Hospital, Ray Brook, New York.

[2] Presented before the Medical Session, as part of *Section 6*, at the annual meeting of the National Tuberculosis Association, Kansas City, Missouri, May 8, 1957.

TABLE 1

INDICATIONS FOR SURGERY

Surgical procedures		70
(a) Resections in the presence of infectious sputum	39	
(b) Resections in the presence of noninfectious sputum	15	
(c) Other	16	
Miscellaneous indications for drug therapy		7
Total indications for drug therapy		77

TABLE 2

RESULTS OF RESECTION IN PATIENTS WITH TUBERCLE BACILLI BY CULTURE IN SPUTUM OR RESECTED TISSUE BY "EFFECTIVE" DRUGS, OPERATIVE PROCEDURE, AND EXTENT OF DISEASE

"Effective" Drugs at Resection	Operations					Early Tuberculous Complications	Late Unsatisfactory Results	Extent of Disease					
								Surgically Treated Lung				Both Lungs N. T. A. Classification	
	Number	P	L	S	W			1+	2+	3+	4+	Moderately Advanced	Far Advanced
Pyrazinamide	21	2	9	5	5	5	8*	4	4	7	6	5	16
Viomycin	1	1					1			1			1
Pyrazinamide and viomycin	12	2	7	3		1	1*		2	4	6	3	9
Pyrazinamide and PAS	4		2	1	1			3		1		2	2
Pyrazinamide, viomycin, and isoniazid	1		1							1			1
Total	39	5	19	9	6	6	10	7	6	14	12	10	29

N.T.A. = National Tuberculosis Association

P = pneumonectomy; L = lobectomy or lobectomy and segmental and/or wedge; S = segmental or segmental and wedge; W = wedge.

* One patient has contralateral cavity.

of the organisms to that drug. Organisms were considered susceptible to pyrazinamide or viomycin if the host had not previously received the drug in question.

In a previous communication (2) it was shown that the amount of pulmonary disease influences both early and late results of pulmonary resection. The same method of classifying the amount of disease in the surgically treated lung is again employed. The extent of disease is indicated as 1- to 4-plus depending upon the estimated volume of lung involved, 1-plus indicating approximately one-quarter and subsequent gradations by quarters up to 4-plus representing disease in the entire lung. Since the amount of disease in the contralateral lung can be expected to affect the late results of surgery, the National Tuberculosis Classification (1955) is also indicated when applicable. It is interesting to note that of the 54 patients included in this series 36 had far advanced and 18 moderately advanced disease.

Pulmonary resections were divided into bacteriologically positive and negative groups depending upon whether or not tubercle bacilli could be cultured from the sputum at the time of surgery or from the resected pulmonary tissue. This was done since it was found that

TABLE 3

RESULTS OF RESECTION IN PATIENTS WITHOUT DEMONSTRABLE TUBERCLE BACILLI BY CULTURE IN SPUTUM OR RESECTED TISSUE BY "EFFECTIVE" DRUGS, OPERATIVE PROCEDURE, AND EXTENT OF DISEASE

"Effective" Drugs at resection	Operations					Early Tuberculous Complications	Late Unsatisfactory Results	Extent of Disease					
								Surgically Treated Lung				Both Lungs N. T. A. Classification	
	Number	P	L	S	W			1+	2+	3+	4+	Moderately Advanced	Far Advanced
Pyrazinamide	1			1					1			1	
Pyrazinamide and viomycin	7		1		6				5	2		2	5
Pyrazinamide and PAS	2			2					2			2	
Pyrazinamide and isoniazid	3			1	2			2	1			3	
Pyrazinamide, viomycin, and isoniazid	1				1				1				1
Pyrazinamide, streptomycin, PAS and isoniazid	1				1					1		1	
Total	15	0	1	4	10	0	0	2	10	3	0	9	6

N. T. A. = National Tuberculosis Association

P = pneumonectomy; L = lobectomy or lobectomy and segmental and/or wedge; S = segmental or segmental and wedge; W = wedge.

early tuberculous complications have not occurred in the bacteriologically "negative" group (3). Moreover, for the purpose of evaluation, the early tuberculous complications (occurring during the first postoperative month) and the late unsatisfactory results were listed. The early tuberculous complications include spread of disease, wound infection, and empyema. The late unsatisfactory results include roentgenographic or bacterial relapse and/or persistently infectious sputum or unfavorable changes in the roentgenogram. Although a patient may have had several relapses, a maximum of one unsatisfactory late result is attributed to each operation.

Surgery other than resection is listed separately in table 4. Since the prognosis was exceedingly poor in many of these patients, it seemed desirable to classify the results less rigidly than in the resections. Accordingly, they were listed as "cured," "improved," and "unimproved." Those patients who achieved sputum negative for tubercle bacilli on culture and cavity closure or obliteration of empyema were classified as cured. Those patients whose cavity or empyema space became smaller were classified as improved. All others were included in the unimproved category. Patients who received single-drug therapy were compared with those who received multiple drugs.

Miscellaneous indications for drug therapy are listed in table 5. The relationship of duration of postoperative drug therapy to early and late results is indicated in table 6. The postoperative drug therapy in these patients represented almost all of the continuous effective drug therapy administered with surgery, since all but 5 patients received these drugs for

TABLE 4

Other Surgery

Procedure	Number	N. T. A. Classification		Results					
		Moderately Advanced	Far Advanced	Single Effective Drug			Multiple Effective Drugs		
				Cured	Improved	Unimproved	Cured	Improved	Unimproved
Decortication of tuberculous empyema	2	1	1	2 (PZA)					
Debridement and closure of tuberculous wound	2		2				1 (PZA + PAS)	1 (PZA + VIO)	
Thoracoplasty									
For cavitary pulmonary tuberculosis	3		3		1 (PZA)			1 (PZA + INH)*	1 (PZA + VIO)†
Bronchopleural fistula and tuberculous empyema	6	1	5	2 (PZA)	2 (PZA)	1 (VIO)‡	1 (PZA + VIO)		
Bronchopleural fistula without empyema	3	1	2	2 (PZA)			1 (PZA + VIO)		
Total	16								

N.T.A. = National Tuberculosis Association.
INH = isoniazid.
PAS = para-aminosalicylic acid.
PZA = pyrazinamide.
VIO = viomycin.

* Thoracoplasty performed after eight months of drug therapy when organisms were resistant to isoniazid and probably pyrazinamide.

† Died from hemorrhage from contralateral cavity (post-mortem confirmation).

‡ Died of progressive disease.

TABLE 5

Miscellaneous Indications for Drug Therapy

Indication	Number	N. T. A. Classification		"Effective" Drugs	Remarks
		Moderately Advanced	Far Advanced		
Post-resection infectious sputum (unexplained)	2	2		PZA and PAS PZA and VIO	Both patients have noninfectious sputum and satisfactory roentgenograms
Tubercle bacilli present in resected specimen resistant to drugs received	1		1	PZA and VIO	Noninfectious sputum and satisfactory roentgenograms
Bronchopleural fistula and empyema (no concomitant surgery)	3		3	PZA (2) PZA and VIO	Unimproved
Monaldi cavernostomy	1		1	PZA and VIO	Noninfectious sputum and satisfactory roentgenograms
Total	7	2	5		

N. T. A. = National Tuberculosis Association
PZA = pyrazinimide.
VIO = viomycin.
PAS = para-aminosalicylic acid.

TABLE 6

RESULTS OF RESECTION IN PATIENTS WITH TUBERCLE BACILLI BY CULTURE IN SPUTUM OR RESECTED TISSUE BY "EFFECTIVE" DRUGS AND DURATION OF DRUG THERAPY

"Effective" Drugs	Number of Operations	Postoperative Drug Therapy in Months							Total		
		0-3	4-6	7-9	10-12	13-15	16-18	19-21	N	E	U
Pyrazinamide	21	N = 5 E = 1 U = 1 E + U = 1		N = 2 (a)	N = 2 U = 1 (b)	N = 1 E + U = 1	U = 2	N = 1 E = 1 (c) U = 1	11	5	8
Viomycin	1	U = 1									1
Pyrazinamide and viomycin	11	N = 1	N = 4 (d, e) U = 1 (f)	N = 2	N = 2		E = 1 (g)		9	1	1
Pyrazinamide and para-aminosalicylic acid	4			N = 2 (h)	N = 2 (i, j)				4		
Pyrazinamide, viomycin, and isoniazid	1				N = 1 (k)				1		
Total	38								25	6	10

N—uncomplicated; E—early tuberculous complication; U—late unsatisfactory result.

(a) One month of viomycin 3 months after operation to cover contralateral operation in one patient.
(b) Contralateral cavity.
(c) Pyrazinamide for 5 months, PAS added one month postoperatively for 19 months.
(d) Cycloserine substituted for viomycin one month postoperatively in one patient.
(e) PAS for pyrazinamide 2 months postoperatively in one patient.
(f) Cycloserine for pyrazinamide 4 months postoperatively; contralateral cavity.
(g) Oxytetracycline for viomycin 9 months postoperatively; 4 months pyrazinamide preoperatively.
(h) Pyrazinamide discontinued after 3 months; isoniazid added after one month in one patient.
(i) Pyrazinamide discontinued after 2 months in one patient.
(j) Pyrazinamide discontinued after 1 month in one patient.
(k) Pyrazinamide discontinued after 8 months.

less than four weeks prior to surgery, and all but 2 patients received these drugs for less than six weeks prior to surgery.

Results

In table 2 it may be seen that, although the patients receiving single effective drug therapy resembled those receiving multiple effective drug therapy in amount of pulmonary disease and type of surgery performed, both early and late results were superior in the latter group. Pulmonary resections performed upon patients without demonstrable tubercle bacilli by culture in the sputum or resected tissue (table 3) were uncomplicated regardless of the drug regimen, type of surgery, or amount of disease.

Procedures other than resection are listed in table 4. Because of the multiplicity of the influencing factors upon the results, no sweeping conclusions are possible. It seems, however, that the best results were obtained in the group of operations which accomplished excision of the major disease (wound debridement and decortication of empyema) or collapse of uninfected pleural space. The results of thoracoplasty for pulmonary disease and tuberculous empyema were satisfactory provided surgery accomplished cavity closure or obliteration of empyema while effective drug therapy was being administered. These were accomplished in the 3 thoracoplasty patients who were cured.

In table 5 it will be noted that multiple-drug therapy was beneficial postoperatively in the treatment of patients who continued to discharge tubercle bacilli in the absence of demonstrable operative complication or persistent pulmonary cavity. The patients who achieved cavity closure by Monaldi cavernostomy (4) also did well. On the other hand, the 3 patients with empyema who received drug therapy unaccompanied by surgery failed to achieve a noninfectious status or obliteration of the pleural space. The evidence in tables 2 through 5 thus indicates that good results can be expected only if intrapulmonary cavities and/or empyema spaces are obliterated by surgery during the period of effective drug therapy.

The influence of the duration of postoperative effective drug therapy upon the early and late results of resection is summarized in table 6. This includes all of the resections with positive evidence of tubercle bacilli by culture in the sputum and resected tissue, with the exception of the patient who died on the seventh postoperative day of hepatic disease. It will be noted that prolonged therapy with a single effective drug did not decrease the incidence of late unsatisfactory results over the incidence of early complications. Indeed, the reverse is true. On the other hand, multiple-drug therapy exerted a beneficial effect upon both early and late results. The high incidence of early and late tuberculous complications which occurred when pyrazinamide was the only effective drug employed may be explained on the basis of the fact that the organisms rapidly developed resistance to the drug. This agrees with the clinical conclusion of Yeager and associates (5) that resistance to pyrazinamide occurred in six to eight weeks. These conclusions were confirmed by Wasz-Höckert and associates (6). On the other hand, the superior results of combined therapy, including viomycin and pyrazinamide, would seem to support the belief that the emergence of drug resistance is delayed when the combinations listed in table 6 are employed.

There has been much evidence published to indicate that this conclusion is valid regarding pyrazinamide and isoniazid (7, 8).

The status of the 54 patients when last examined is indicated in table 7. It is not surprising that the patients who received multiple-drug therapy in conjunction with pulmonary resection have achieved the best results. With the exception of the patient who died of hepatic disease which could have represented drug toxicity, the only patient in this group who has not achieved a satisfactory result has a cavity in the lung contralateral to the one operated upon.

Drug toxicity severe enough to necessitate cessation of therapy is summarized in table 8. Pyrazinamide was discontinued because of increasing bromsulphalein (BSP) retention in 3 patients and the occurrence of jaundice in 2 patients. One

TABLE 7

STATUS OF PATIENTS UPON LAST EXAMINATION

Indication*	Number of Patients	Satisfactory Roentgenogram and Sputum Negative for Tubercle Bacilli	Unsatisfactory Roentgenogram and/or Sputum Positive for Tubercle Bacilli	Dead
Resections with positive bacteriology				
One drug	20	16	4†	
Multiple drugs	16	14	1†	1‡
Resections with negative bacteriology	7	7		
Other	11	4	5	2
Total	54			

* Each patient is listed according to primary indication for drug therapy even though more than one indication might have existed in the same individual.

† One of these patients had contralateral cavity.

‡ Died probably of drug toxicity.

TABLE 8

TOXICITY REQUIRING CESSATION OF DRUG THERAPY

Drug	Reason	Number	Remarks
Pyrazinamide	Elevated bromsulphalein	3	1 had fever. 1 had chronic pancreatitis.
	Jaundice	2	1 appeared 2 months post-operatively (?homologous serum hepatitis). 1 died; focal hepatic necrosis.
Viomycin	Severe "allergy" (nausea, vomiting, diarrhea, erythema, dizziness)	2	1 had transient decrease in blood pressure.
	Progressive loss of hearing (audiometry)	1	

of the latter may have had homologous serum hepatitis. The other was a 48-year-old male with Laennec's cirrhosis, who was an alcoholic. Approximately twenty-eight days after initiation of pyrazinamide and viomycin therapy and six days after pulmonary resection, the patient developed jaundice and died about twenty-four hours later. Post-mortem examination revealed the Laennec's cirrhosis and "recent parenchymatous and vacuolar degeneration of the liver."

There were no tuberculous postoperative complications. Several patients who received pyrazinamide complained of vague pains in the joints, especially the small joints of the hand. Although blood uric acid levels were not determined, it appears likely that the symptoms might have been due to "hyperuricemia" (9).

Viomycin was discontinued in two instances because of nausea, vomiting, diarrhea, erythema, and/or dizziness. One patient experienced a decrease in blood pressure which did not return to normal for several hours. Several patients who experienced similar symptoms to a milder degree have been able subsequently to tolerate the drug with little difficulty. One patient developed progressive loss of hearing for the higher audible frequencies as determined by audiometry.

Discussion

The evidence obtained from this series of patients indicates that the results of surgical resection performed under the protection of pyrazinamide in combination with viomycin or PAS, and probably other antituberculous drugs, are superior to the results obtained with pyrazinamide alone. The results of such multiple-drug therapy also appear superior to the reported results of viomycin alone (10). The comparatively low incidence of late unsatisfactory results in the group of patients with resections who received multiple-drug therapy would seem to support the impression that drug resistance was delayed beyond the period when it might have emerged had each drug been used alone.

Since such multiple-drug therapy seems to offer prolonged protection, an attempt has been made to employ two drugs to cover surgery whenever possible. In addition to the combinations reported here, regimens including cycloserine, thiosemicarbazone and a sulfone are being evaluated.

When planning pulmonary resection it would seem advisable to substitute for existing drug regimens two new drugs or two drugs to which the patient's tubercle bacilli were known to be susceptible when:

(*1*) The tubercle bacilli discharged in the sputum are known to be resistant to the existing drug regimen.

(*2*) Tubercle bacilli are present in the sputum and the patient has received drugs intermittently or for many months.

(*3*) Tubercle bacilli cannot be demonstrated in the sputum but the organisms were resistant to the currently administered drugs prior to the reversal of infectiousness.

(*4*) The discharge of tubercle bacilli in the sputum has recently ceased but the patient has been receiving the drugs currently employed intermittently over a prolonged period, and the lung to be operated upon contains a large amount of disease.

(*5*) Postoperatively, the resected specimen yields tubercle bacilli resistant

to the current drug regimen, especially if a large amount of disease was present in the lung operated upon.

Finally, in this connection it should be emphasized that good surgical results depend upon factors other than adequate drug therapy. Some of these factors have been discussed elsewhere (3).

It is important to note that the best results in the present series were obtained in the group of operations which accomplished excision or obliteration of pulmonary cavities, empyema spaces, or infected wounds. Persistent pulmonary cavities or empyema spaces were not affected by any of the drug regimens employed. Therefore, it appears important to combine effective drug therapy with effective surgery. Moreover, surgery should be performed early in the course of drug therapy, before the tubercle bacilli have developed resistance to the drugs administered. It should be emphasized, however, that this program is advocated only in the treatment of patients known to be harboring, or suspected of harboring, tubercle bacilli resistant to streptomycin, PAS, and/or isoniazid, since many of these patients have already demonstrated their lack of favorable response to drug therapy without surgery. For patients receiving their initial treatment and for many re-treatment patients whose tubercle bacilli are susceptible to streptomycin, PAS, and isoniazid, it is still considered preferable to delay surgery until maximal response to drug therapy has been achieved.

Another reason for administering pyrazinamide and viomycin for a short time preoperatively is the relatively high toxicity of these drugs, which sometime precludes prolonged therapy. Occasionally, rather than continue single-drug therapy, other drugs (such as oxytetracycline, cycloserine, thiosemicarbazone, and a sulfone) were substituted for the toxic drug.

Summary

Experience with pyrazinamide and viomycin in the treatment of surgical conditions is reviewed. The drugs were used in connection with 70 surgical procedures and in the treatment of 7 patients who had no concomitant surgery. Multiple-drug therapy was found to be superior to single-drug therapy. When intrapulmonary cavity or pleural empyema were present, the best results were obtained when surgical excision or collapse were combined with effective drug therapy.

Sumario

La Piracinamida y la Viomicina en el Tratamiento Quirúrgico de la Tuberculosis Pulmonar

Repásase lo observado con la piracinamida y la viomicina en el tratamiento de estados quirúrgicos. Las drogas fueron usadas en relación con 70 procedimientos quirúrgicos y en el tratamiento de 7 enfermos que no recibieron cirugía concomitante. La terapéutica con varias drogas resultó superior a la terapéutica con una sola droga. Cuando existían caverna intrapulmonar o empiema pleural, se obtuvieron los mejores resultados cuando se combinaban la excisión o el colapso quirúrgicos con una farmacoterapia eficaz.

Resume

Pyrazinamide et Viomycine dans le traitement chirurgical de la tuberculose pulmonaire

Compte-rendu de l'expérience acquise avec la Pyrazinamide et la Viomycine dans le traitement des cas chirurgicaux. Ces drogues furent utilisées en relation avec 70 interven-

tions chirurgicales et dans le traitement de 7 malades non soumis à une intervention chirurgicale concomitante. Le traitement par les associations médicamenteuses a été trouvé supérieur au traitement avec un médicament unique. Quand l'excavation intrapulmonaire ou l'empyème pleural existait, les meilleurs résultats furent obtenus quand une chimiothérapie adéquate était associée à l'excision chirurgicale ou au collapsus.

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THE EFFECT OF DIET ON THE INDUCEMENT OF ACQUIRED RESISTANCE BY VIABLE AND NONVIABLE VACCINES IN EXPERIMENTAL TUBERCULOSIS[1, 2]

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(Received for publication June 21, 1957)

Introduction

In a previous investigation it was demonstrated that the administration to mice of a synthetic ration which contained a group of fatty acid esters, mixed in proportions to simulate coconut oil, increased the resistance of the animals to infection with the H37Rv strain of *Mycobacterium tuberculosis* (1). Enhanced resistance of the animals was manifested as an abrupt decrease in the rate of death which occurred two to three weeks following the injection of the tubercle bacilli. Since the mice became refractory only after the organisms had been in the tissues for two weeks or longer, it was postulated that the mechanisms of the host which were responsible for the development of acquired resistance to tuberculosis were activated and stimulated by the fatty acids in the diet. In the present paper a determination has been made of the effect of the synthetic ration containing selected fatty acids on the development of acquired resistance to tuberculosis in mice following the injection of viable and nonviable suspensions of the BCG strain and a nonviable suspension of the H37Rv strain of *M. tuberculosis*.

Materials and Methods

Mice: Male and female CF_1 mice (14 to 16 gm. each) were employed as test animals. The method employed in maintenance of the animals has been described previously (1).

Diets: The basal diet utilized for incorporation of fatty acids was identical to that used previously (1). Rockland Mouse Diet was used as the control ration. All rations were furnished ad libitum to the mice.

The three mixtures of fatty acid esters which were used in this investigation are presented in table 1. The first mixture, which has been designated fatty acids No. 1, was identical to that previously reported (1). The second and third fatty acid mixtures, designated fatty acids Nos. 2 and 3, contained increased amounts of unsaturated fatty acids.

Methyl laurate, methyl myristate, methyl stearate, methyl oleate,[3] methyl palmitate,[4] and methyl linoleate[5] were purified by fractional distillation under reduced pressure.

Organisms: The H37Rv strain of *M. tuberculosis* (2) was used as the infective agent in this study. For use in the experiments, the organisms were cultured from splenic tissue which had been removed aseptically from control groups of mice used in previous experiments and stored in 0.1 per cent bovine albumin at −20°C. The initial culture was made in Kirch-

[1] Presented in part at the medical session, as part of *Section 4B*, of the annual meeting of the National Tuberculosis Association, Kansas City, Missouri, May 7, 1957.

[2] From the Research Laboratory, Veterans Administration Hospital, Kansas City, Missouri; and the Department of Medical Microbiology, University of Kansas Medical Center, Kansas City, Kansas.

[3] Manufactured by Matheson, Coleman and Bell Chemicals Co.

[4] Manufactured by Eastman Kodak Co.

[5] Manufactured by Nutritional Biochemicals Co.

TABLE 1

COMPOSITION OF MIXTURES OF FATTY ACIDS

Fatty Acid Mixtures	Methyl Laurate	Methyl Myristate	Methyl Palmitate	Methyl Stearate	Methyl Oleate	Methyl Linoleate
Fatty acids No. 1	50	25	20	2	2	1
Fatty acids No. 2	25	15	25	8	22	5
Fatty acids No. 3	0	0	8	4	40	48

ner medium containing Tween® 80 and bovine albumin. After two subcultures in this medium the tubercle bacilli were cultured for five days in Kirchner medium and Tween 80 without albumin. These organisms were used for preparation of a nonviable vaccine and also for infection of the mice.

The BCG "Phipps" strain of attenuated *M. tuberculosis*, variety *bovis*, was obtained from Dr. J. Aronson, Henry Phipps Institute, Philadelphia. The organisms were cultured in Dubos medium containing Tween 80 and albumin (3, 4).

Immunization and infection of animals: For immunization of the animals with viable BCG, six-day cultures were diluted in Dubos medium without albumin until the concentration of organisms reached 4×10^6 viable units per ml. of medium. A 1:10 dilution of a suspension having a transmittance of 67 per cent at 640[6] was found by plate counts to contain 4×10^6 viable units per ml. Each mouse was injected intraperitoneally with 0.2 ml. of the culture diluted in Dubos medium. In preparation of a nonviable vaccine, five-day cultures of H37Rv in Kirchner medium (or BCG in Dubos medium) were transferred to a 50 ml. screw-cap test tube and cooled in an ice bath. Ethylene oxide, in the liquid phase, was then added in a concentration of 1 per cent of the culture (5). After the caps had been tightened, the organisms were held at 6°C. for one hour, then transferred to the incubator and maintained at 37°C. for twenty-four hours with loosened caps. Sterility of the treated cultures was ascertained by inoculation on Löwenstein medium. For use as a vaccine the nonviable cell suspension was diluted in sterile Dubos medium until the concentration reached 4×10^6 cellular units per ml. Each mouse was injected intraperitoneally with 0.2 ml. of the vaccines.

All of the mice were fed commercial "chow" for seven days following their arrival. The animals were then segregated into groups and fed their respective rations. Appropriate groups of animals also received the various vaccine preparations at the time of segregation. Five to eleven days after the mice were segregated and vaccinated, all of the animals were challenged by the intravenous injection of 0.1 ml. of a suitable concentration of a five-day culture of H37Rv.

RESULTS

In the initial experiment a determination was made of the effect of the administration of the following rations on the rate of death of groups of male mice (14 per group):

(*1*) Synthetic ration with 5 per cent fatty acids No. 1; (*2*) synthetic ration with 5 per cent fatty acids No. 2; (*3*) commercial "chow." The rations were administered for ten days. At this time each animal was injected intravenously with 0.1 ml. of a five-day culture of *M. tuberculosis* diluted with an equal amount of Kirchner medium. The deaths in each group of animals were recorded daily for fifty-eight days, at which time all of the animals in *Group 3* had succumbed.

[6] Measured in the Rouy photrometer.

The rate of death of the animals in *Group 3* followed a normal frequency distribution curve and the average mortality time was twenty-seven days (figure 1). In the groups of animals which had been fed the synthetic rations with fatty acids Nos. 1 and 2, an abrupt decrease in the rate of death occurred twenty-five to thirty-five days following infection. At the termination of the experiment (fifty-eight days after infection), there was a mortality of 50 per cent in *Group 1* and of 21 per cent in *Group 2*.

To determine whether the diet of the mice affected the immune response of the animals, a determination was made of the effectiveness of BCG in enhancing the resistance of groups of male mice fed the synthetic ration with fatty acids No. 1 and commercial "chow".

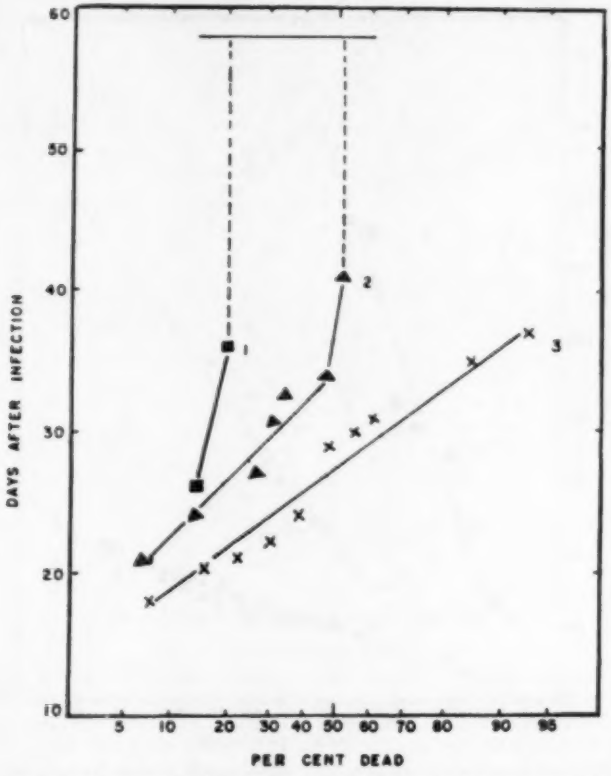

FIG. 1. Effect of dietary fatty acids on rate of death of mice (14 per group) infected with *M. tuberculosis*. Curve 1, synthetic ration, 5 per cent fatty acids No. 2; curve 2, synthetic ration, 5 per cent fatty acids No. 1; curve 3, commercial "chow."

Two groups of mice were fed commercial "chow," one of which received viable BCG. The synthetic ration containing fatty acid esters was administered also to two groups of mice, one of which received BCG. The mice (20 per group) were placed on their rations and injected with the BCG ten days prior to infection. The animals were injected with 0.1 ml. of a five-day culture of *M. tuberculosis* diluted with an equal amount of Kirchner medium. The experiment was terminated one hundred and thirty-five days after infection. At this time all of the animals in the control groups as well as those in the group which had received BCG and commercial "chow" had succumbed (figure 2).

Only 35 per cent of the mice that had been injected with BCG and fed the synthetic ration with fatty acids succumbed during the experiment (figure 2). The unvaccinated animals which had been fed the synthetic ration died at the same rate as the controls. It is not known why the synthetic ration with fatty acids No. 1 did not effect the expected decline in the rate of death of the un

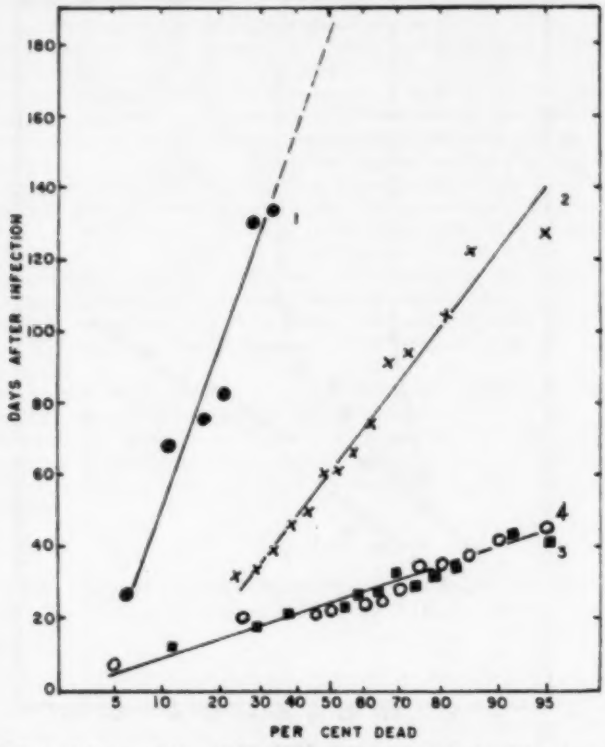

FIG. 2. Effect of dietary fatty acids on the rate of death of mice (20 per group) injected with BCG ten days prior to infection with *M. tuberculosis*. Curve 1, animals injected with BCG and fed synthetic ration with 5 per cent fatty acids No. 1; curve 2, mice injected with BCG and fed commercial "chow"; curve 3, animals not injected with BCG and fed commercial "chow;" curve 4, animals not injected with BCG and fed synthetic ration with fatty acids No. 1.

vaccinated mice. With respect to the infecting organism, however, it has been found that, upon repeated transfer *in vitro*, this strain of H37Rv retains its virulence but produces infections in mice which respond atypically to dietary fatty acids.

The effect of diet on the development of acquired resistance to tuberculosis was explored further in view of the increased resistance displayed by those

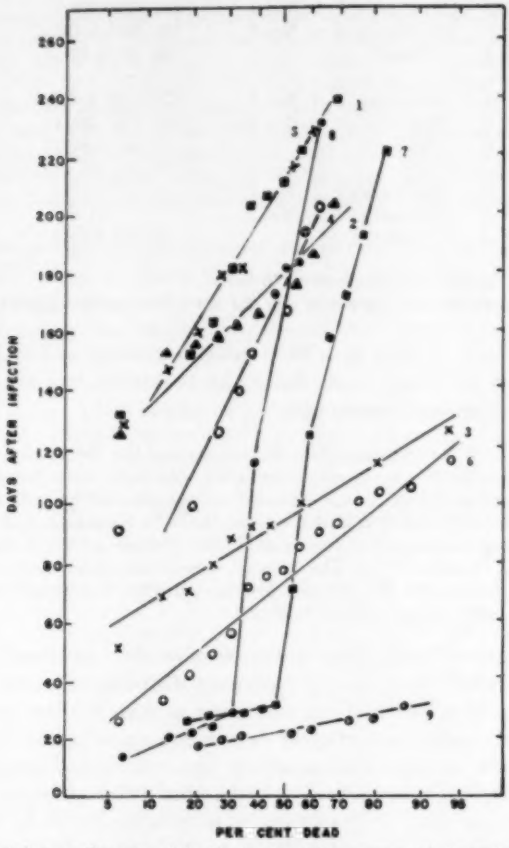

FIG. 3. Effect of diet on the rate of death of mice (16 per group) injected with viable BCG or nonviable H37Rv eleven days prior to infection with *M. tuberculosis*. Curve 1, injected with BCG and fed synthetic ration with fatty acids No. 1; curve 2, injected with BCG and fed synthetic ration with fatty acids No. 2; curve 3, injected with BCG and fed commercial "chow"; curve 4, injected with nonviable H37Rv and fed synthetic ration with fatty acids No. 1; curve 5, injected with nonviable H37Rv and fed synthetic ration with fatty acids No. 2; curve 6, injected with nonviable H37Rv and fed commercial "chow"; curve 7, not vaccinated and fed synthetic ration with fatty acids No. 1; curve 8, not vaccinated and fed synthetic ration with fatty acids No. 2; curve 9, not vaccinated and fed commercial "chow."

TABLE 2

EFFECT OF DIET ON THE DEVELOPMENT OF ACQUIRED RESISTANCE FOLLOWING INJECTION OF VIABLE BCG AND NONVIABLE H37Rv ELEVEN DAYS PRIOR TO INFECTION

Immunizing Agent	Animal* Group	Ration	T_{50} and 95 Per Cent Confidence Limits	Per Cent Surviving 240 Days after Infection
			days	
Viable BCG	1	Synthetic, F.A. No. 1	214 (193–236.7)	31.2
	2	Synthetic, F.A. No. 2	180 (167.5–193.2)	31.2
	3	"Chow"	94 (86.4–104.5)	0
Nonviable H37Rv	4	Synthetic, F.A. No. 1	176 (149.2–207.6)	25.0
	5	Synthetic, F.A. No. 2	214 (193–236.7)	43.7
	6	"Chow"	74 (64.3–85.2)	0
None	7	Synthetic, F.A. No. 1	—†	18.8
	8	Synthetic, F.A. No. 2	—†	37.5
	9	"Chow"	22 (19.7–24.6)	0

* Each animal group was composed of 16 mice.

† Value not included since rate of death did not follow normal distribution curve.

animals which were injected with BCG prior to infection and maintained on the synthetic ration with fatty acids No. 1. An evaluation was also made of nonviable H37Rv as an immunizing agent.

In this experiment the following three diets comprised the dietary groups: (*a*) synthetic ration with fatty acids No. 1; (*b*) synthetic ration with fatty acids No. 2; (*c*) commercial "chow." Three groups of female mice (16 each) were maintained on each of the rations and treated as follows: (*1*) animals injected with viable BCG (Groups *1, 2, 3*); (*2*) animals injected with a vaccine composed of nonviable H37Rv (*Groups 4, 5 6*); (*3*) animals untreated prior to infection (*Groups 7, 8, 9*). The vaccines were administered eleven days prior to infection with *M. tuberculosis* (0.1 ml. of a five-day culture). The results and evaluation of the data are recorded in figure 3 and table 2.

While the rate of death of the untreated animals maintained on commercial "chow" corresponded to a normal frequency distribution curve, an abrupt decrease in the rate of death of the untreated animals fed the synthetic rations occurred twenty-eight to thirty-one days following infection. The decrease in the rate of death occurred simultaneously in unvaccinated groups fed the synthetic rations, but a larger percentage of animals were resistant (after twenty-eight to thirty-one days) in the group fed fatty acids No. 2. At the termination of the experiment, 240 days after infection, the existing mortality was 81.2 per cent in the animals which had received fatty acids No. 1 and 62.5 per cent in those fed fatty acids No. 2. The rate of death of the animals in all other groups corresponded to a normal frequency distribution curve. The average mortality times of groups of mice which had received viable BCG or nonviable H37Rv and were fed the synthetic ration with fatty acids Nos. 1 or 2 were two to two and a half times greater than the mortality times of similarly treated animals maintained on commercial "chow" and eight to nine times greater than those

of the untreated animals fed commercial "chow." Viable BCG and nonviable H37Rv yielded similar results as immunizing agents.

The next experiment was designed to study the effect of rations which contained predominantly unsaturated fatty acids (fatty acids No. 3) on the development of acquired resistance as well as to confirm the results of the previous experiment.

The dietary groups consisted of: (*a*) synthetic ration with fatty acids No. 2; (*b*) synthetic ration with fatty acids No. 3; (*c*) commercial "chow." Three groups of male mice were fed each of the rations and each group was treated as follows: (*1*) animals injected with viable BCG (*Groups 1, 2, 3*); (*2*) animals injected with a vaccine composed of nonviable H37Rv (*Groups 4, 5, 6*); (*3*) animals untreated prior to infection (*Groups 7, 8, 9*). The vaccines were administered six days prior to infection. The animals were maintained on the rations for ten days. Each animal then was injected with 0.1 ml. of a five-day culture of *M. tuberculosis*. The mortality data for each group of animals were recorded daily during the experimental period of 243 days (table 3), at which time the experiment was terminated.

In all of the groups of unvaccinated animals the rate of death decreased four weeks after infection, which was minimal in the animals fed "chow" but very marked in the groups of animals fed the two synthetic rations. More animals in the unvaccinated group which had received the synthetic ration with fatty

TABLE 3

EFFECT OF DIET ON THE DEVELOPMENT OF ACQUIRED RESISTANCE FOLLOWING INJECTION OF VIABLE BCG AND NONVIABLE H37Rv SIX DAYS PRIOR TO INFECTION WITH MYCOBACTERIUM TUBERCULOSIS

Immunizing Agent	Animal Group	Number of Mice per Group	Ration	Survival Times of Mice After Challenging Infection	Median Survival Time
				days	*days*
Viable BCG	1	15	Synthetic, F.A. No. 2	59, 90, 120, 129, 180, 180, 181, 181, 182, S, S, S, S, S, S	181
	2	15	Synthetic, F.A. No. 3	97, 106, 131, 149, 213, 214, 214, 214, 215, 215, 215, S, S, S, S	214
	3	15	"Chow"	25, 47, 55, 68, 68, 75, 78, 78, 82, 90, 95, 102, 102, 106, 115	78
Nonviable H37Rv	4	14	Synthetic, F.A. No. 2	32, 60, 60, 80, 139, 180, 216, 216, 216, 219, S, S, S, S	216
	5	14	Synthetic, F.A. No. 3	64, 106, 128, 150, 184, 194, 202, 211, 212, 213, S, S, S, S	202
	6	14	"Chow"	38, 53, 69, 82, 82, 85, 85, 85, 85, 85, 86, 86, 87, 118	85
None	7	14	Synthetic, F.A. No. 2	22, 25, 35, 50, 90, 140, 140, 141, 141, 141, 143, 197, S, S	140
	8	16	Synthetic, F.A. No. 3	16, 19, 21, 22, 25, 25, 26, 26, 28, 62, 83, 141, 142, 142, 150, 151	26
	9	14	"Chow"	15, 18, 20, 23, 24, 24, 27, 35, 41, 51, 58, 69, 78, 122	27

acids No. 2 had acquired resistance four weeks after infection than in the group which had been fed the synthetic ration containing fatty acids No. 3. Viable BCG and nonviable H37Rv were equivalent as immunizing agents. A similar degree of resistance was effected in the vaccinated animals fed the synthetic ration with fatty acids Nos. 2 and 3. The resistance was twice as great in the vaccinated mice fed the synthetic ration as in similarly treated animals which had been fed "chow," and seven to eight times greater than in unvaccinated mice maintained on "chow."

A subsequent experiment was designed for the following purposes: to compare nonviable BCG with nonviable H37Rv and viable BCG as immunizing agents in mice under varied dietary conditions; to establish the effect of the synthetic ration containing only a minimal amount of an essential fatty acid (0.2 per cent methyl linoleate) on the development of acquired resistance to tuberculosis; and to determine if a further reduction in the length of the period during which the animals were vaccinated and maintained on their rations prior to infection would make apparent any acceleration of immune response which resulted from the varied dietary regiments.

The dietary groups consisted of: (a) synthetic ration with fatty acids No. 2, (b) synthetic ration with 0.2 per cent methyl linoleate, (c) commercial "chow." Four groups of male mice were maintained on each of the rations and were treated as follows: (*1*) animals injected

TABLE 4

EFFECT OF DIET ON THE DEVELOPMENT OF ACQUIRED RESISTANCE FOLLOWING INJECTION OF VIABLE AND NONVIABLE BCG AND NONVIABLE H37Rv FIVE DAYS PRIOR TO INFECTION WITH MYCOBACTERIUM TUBERCULOSIS

Immunizing Agent	Animal Group	Number of Mice per Group	Ration	T_{50} and 95 Per Cent Confidence Limits	Number of Mice Surviving 120 Days after Infection
				days	
Viable BCG	1	16	Synthetic, F.A. No. 2	—*	9
	2	16	Synthetic, Methyl linoleate	74.5 (58.6–94.6)	0
	3	16	"Chow"	28.1 (24.7–32.1)	0
Nonviable BCG	4	16	Synthetic, F.A. No. 2	—*	13
	5	17	Synthetic, Methyl linoleate	86.5 (67.6–110.7)	2
	6	17	"Chow"	28 (25.9–30.3)	0
Nonviable H37Rv	7	17	Synthetic, F.A. No. 2	—*	13
	8	17	Synthetic, Methyl linoleate	79 (59.4–105.1)	2
	9	17	"Chow"	34 (26.7–43.4)	0
None	10	17	Synthetic, F.A. No. 2	—†	5
	11	17	Synthetic, Methyl linoleate	—†	0
	12	17	"Chow"	23 (21–25.1)	0

* T_{50} not attained due to limited mortality.

† Value not included since rate of death did not follow normal distribution curve.

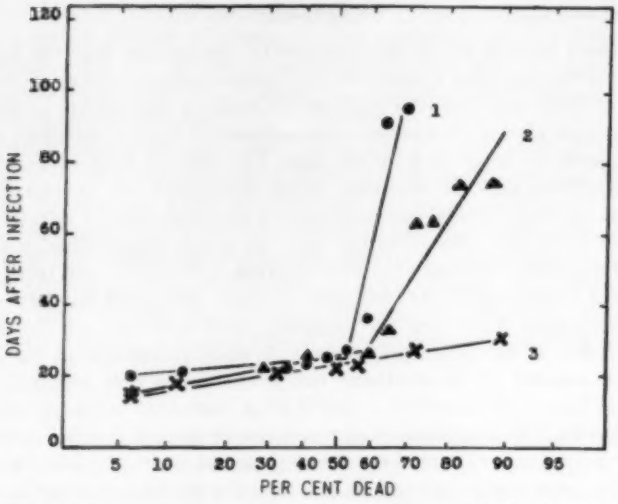

FIG. 4. Effect of diet on the rate of death of mice infected with *M. tuberculosis*. Curve 1 synthetic ration, 5 per cent fatty acids No. 2; curve 2, synthetic ration, 0.2 per cent methy linoleate; curve 3, commercial "chow."

with viable BCG (*Groups 1, 2, 3*); (*2*) animals injected with a vaccine composed of nonviable BCG (*Groups 4, 5, 6*); (*3*) animals injected with a vaccine composed of nonviable H37Rv (*Groups 7, 8, 9*); (*4*) animals untreated prior to infection (*Groups 10, 11, 12*). The mice were vaccinated and placed on their rations five days prior to infection. Each animal was infected by the 0.1 ml. injection of a five-day culture of H37Rv. The results of the experiment, which was terminated 120 days after infection, are recorded in table 4 and figure 4.

In the group of unvaccinated animals fed the synthetic ration with fatty acids No. 2, resistance was established in 47 per cent of the mice four weeks after infection. Twenty-nine and four-tenths per cent of the animals survived until the termination of the experiment. A lower level of resistance was also established in 41 per cent of the unvaccinated mice which received the synthetic ration with methyl linoleate. After ninety-seven days, however, all of the mice fed the synthetic ration with methyl linoleate had succumbed. The rate of death of the mice in each of the other groups followed a normal distribution curve. There was very little difference in the rates of death of the vaccinated and unvaccinated mice which had been fed commercial "chow." The groups of animals fed the synthetic ration with fatty acids No. 2 acquired a high level of resistance. While the activity of the vaccines was reduced, the average mortality times of groups of vaccinated mice fed the synthetic ration with methyl linoleate were three to four times greater than the unvaccinated animals fed "chow." Viable BCG, nonviable BCG, and nonviable H37Rv produced similar results as immunizing agents.

Discussion

The present findings are in agreement with the concept that the fatty acids in the diet of the host play a part in the activation and stimulation of the mechanisms involved in acquired resistance to tuberculosis. The ability of the animal host to acquire a state of resistance immediately following infection appeared to be regulated by fatty acids in the diet. The type of fatty acids which had been incorporated into the synthetic ration determined the percentage of animals which acquired enough resistance during the early stage of infection so that the disease followed a chronic rather than an acute, rapidly fatal course. The fatty acid mixtures were active in the following order: fatty acid mixture No. 2 (73 per cent saturated, 27 per cent unsaturated); fatty acid mixture No. 1 (97 per cent saturated, 3 per cent unsaturated); fatty acid mixture No. 3 (12 per cent saturated, 88 per cent unsaturated). A small percentage of unvaccinated animals maintained on the synthetic ration containing only minimal amounts of essential fatty acids acquired a low level of resistance following injection of tubercle bacilli. The development of resistance in certain animals in this group may be related to the fact that the five-day period of feeding prior to infection (used in this experiment) was insufficient time for the complete depletion of the preformed body fat of the mice (6).

While the dietary fatty acids appeared to regulate the ability of the mice to acquire resistance immediately following infection, the data indicate that another unknown dietary factor (s) was largely responsible for determining the degree of immunity attained, since the type of fatty acid mixture added to the synthetic ration when the concentration remained at 5 per cent did not alter the degree of resistance ultimately acquired by the vaccinated and unvaccinated animals. The effectiveness of the vaccines was reduced when the fatty acid content of the animal ration was reduced to 0.2 per cent, but the short five-day period of immunization may have been insufficient for the development of maximal resistance under the prevailing dietary conditions. The ability of the synthetic ration with appropriate fatty acids to accelerate as well as intensify the mechanisms involved in resistance of the host is expressed in the final experiment in which the period of immunization was restricted by administering the vaccines (and rations) five days prior to infection. Under these conditions the vaccinated animals which were maintained on commercial "chow" failed to acquire resistance, whereas a high level of resistance was acquired by similarly treated animals fed the synthetic ration with fatty acids No. 2.

Under the conditions of the present investigation either nonviable BCG or nonviable H37Rv was as effective as viable BCG in inducing resistance to tuberculosis (in terms of amount of cellular material introduced and the degree of resistance attained). The results are in partial agreement with those of Swedburg (7), Solotorovsky and associates (8), and Dubos and associates (9) who found that nonviable organisms were effective in inducing immunity. On the other hand, these investigators found that the injection of nonviable cells failed to establish a degree of resistance equivalent to that produced by viable cells. The results are at variance with those of Bloch and Segal (10). The latter in-

vestigators reported that the immunizing effectiveness of a vaccine was dependent upon the number of living cells present. This conclusion was based upon a study of the effectiveness of vaccines prepared by exposing BCG to ultraviolet light for varied intervals of time after which the preparations were evaluated by intravenous injection into mice.

The effectiveness of the nonviable vaccines used in this study may have been due, in part, to the use of ethylene oxide for killing the H37Rv and BCG strains composing the vaccines. Ethylene oxide was utilized for this purpose, since it is a potent lethal agent and is very convenient to use. The mode of action of ethylene oxide on microorganisms is unknown; however, the agent has been found to react with most of the available reactive groups in protein, e.g., carboxyl, amino, sulphydryl, and phenol (11). Exposure of protein to ethylene oxide for long periods of time results in destruction of large amounts of methionine and histidine (12).

Summary

The resistance of vaccinated and unvaccinated CF_1 mice to tuberculous infection was increased by the administration of a synthetic ration which contained mixtures of fatty acid esters added in a concentration of 5 per cent. In the unvaccinated groups of mice fed the synthetic ration with fatty acids No. 2 (73 per cent saturated, 27 per cent unsaturated), 70 to 80 per cent of the tuberculous animals acquired sufficient resistance during the early stage of infection so that the disease followed a chronic rather than an acute course. Fewer animals developed resistance during the early stage of infection when other fatty acid mixtures replaced fatty acids No. 2 in synthetic ration. A comparable degree of resistance did not develop in groups of unvaccinated mice which were fed commercial "chow."

The resistance of mice (in terms of survival time) vaccinated with viable and nonviable BCG or nonviable H37Rv and fed the synthetic ration with the fatty acid mixtures was two- to threefold greater than that of vaccinated mice fed commercial "chow" and eight- to ninefold greater than that of the unvaccinated animals which were fed "chow." The degree of resistance acquired by the vaccinated animals which received the synthetic ration was unaffected by variation in concentration of the five individual fatty acids composing the mixtures when the total concentration remained at 5 per cent.

Suspensions of H37Rv or BCG which had been killed by treatment with ethylene oxide were as effective as viable BCG in inducing resistance to tuberculosis in CF_1 mice, both in terms of amount of antigenic cellular material introduced and in the degree of resistance attained.

Sumario

El Efecto de la Alimentación sobre la Inducción de Resistencia Adquirida por Vacunas Viables y Aviables en la Tuberculosis Experimental

La resistencia de los ratones CF_1 vacunados y no vacunados a la infección tuberculosa aumentó al administrárseles una ración sintética que contenía mezclas de ésteres de ácidos grasos agregados a una concentración de 5 por ciento. En los grupos de ratones no vacunados

que recibieron la ración sintética con ácidos grasos No. 2 (73 por ciento saturados, 27 por ciento sin saturar), de 70 a 80 por ciento de los animales tuberculosos adquirieron suficiente resistencia durante la etapa incipiente de la infección, de modo que la enfermedad manifestó evolución cronica más que aguda. Cuando mezclas de otros ácidos grasos suplantaron a los de No. 2 en la ración sintética, menos animales manifestaron resistencia durante el período incipiente de la infección. No apareció resistencia de grado comparable en los grupos de ratones sin vacunar que recibieron alimento obtenido en el comercio.

La resistencia de los ratones (en términos de tiempo de sobrevivencia) vacunados con BCG viables y aviables o con H37Rv aviables y que recibieron la ración sintética fué de dos a tres veces mayor que la de los ratones vacunados que recibieron alimento obtenido en el comercio y de ocho a nueve veces mayor que la de los animals sin vacunar que recibieron dicho alimento. La intensidad de la resistencia adquirida por los animales vacunados que recibieron la ración sintética no se vió afectada por la variación en la concentración de los cinco distintos ácidos grasos que componían las mezclas si la concentración total se mantenía a 5 por ciento.

Las suspensiones de H37Rv o BCG que habían sido matados por tratamiento con óxido de etileno se mostraron tan eficaces como los BCG viables en lo tocante a inducir resistencia a la tuberculosis en los ratones CF_1, en términos tanto de la cantidad de materias celulares antigénicas introducidas como de la intensidad de la resistencia alcanzada.

Resume

Les effets du régime alimentaire sur l'introduction de la résistance acquise au moyen de vaccins viables et non-viables dans la tuberculose expérimentale

La résistance à la tuberculose des souris CF_1 vaccinées et non vaccinées à été augmentée par l'administration d'une ration synthétique renfermant 5% d'un mélange d'esters d'acide gras. Dans les groupes de souris non vaccinées recevant la ration synthétique avec les acides gras No. 2. (73% saturés, 27% non saturés), 70 à 80% des animaux tuberculisés acquérirent une résistance suffisante durant le stade initial de l'infection, de sorte que la maladie suivait un cours chronique plutôt qu'aigu. Lorsque d'autres mélanges d'acides gras remplacèrent les acides gras No. 2 dans la ration synthétique, il y eut moins d'animaux qui développèrent une résistance au stade initial de l'infection. Un degré de résistance comparable ne s'est pas manifesté dans les groupes de souris non vaccinées nourries avec les aliments ordinairement en vente.

La résistance des souris (en terme de temps de survie) vaccinées avec le BCG viable et non viable ou avec le H37Rv non viable et nourries avec la ration synthétique comportant les mélanges d'acides gras était de deux à trois fois plus grande que celle des souris vaccinées nourries avec les aliments ordinairement en vente et huit à neuf fois plus grande que celle des animaux non vaccinés nourris avec les aliments ordinaires. Le degré de résistance acquise par les animaux vaccinés recevant la ration synthétique n'était pas modifié par la variation de la concentration des cinq acides gras particuliers composant les mélanges, quand la concentration totale se maintenait à 5%.

Les suspensions de H37Rv ou de BCG tué par le traitement avec l'oxyde d'éthylène étaient aussi efficaces que le BCG viable pour susciter la résistance à la tuberculose chez les souris CF_1, à la fois en termes de substance cellulaire antigénique introduite et quant au degré de résistance atteint.

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PROTEIN AND NUCLEIC ACID IN CASEOUS NECROSIS[1]

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(Received for publication July 31, 1937)

INTRODUCTION

Dead and dying cells may contain abundant protein despite gross losses of other cellular constituents (1–3). Of all agents capable of killing cells, *M. tuberculosis* is one of the most potent in causing extensive lethal injury. The present communication gives an account of some cytochemical features of tuberculous caseation in an attempt to follow the fate of certain major constituents of dying cells during caseous necrosis.

The distribution of nucleic acids in the cells of human tuberculous granulation tissue was studied by Monaci and Coppitz (4). These workers noted that caseous material is on the whole uncolored by the Feulgen method for desoxyribonucleic acid, but contains some colored nuclear remnants. They also observed coloration of the nuclei of giant cells and epithelioid cells, and they state that the nuclei of the latter contain but little desoxyribonucleic acid; ribonucleic acid, colored by pyronine, was found in the cytoplasm of both epithelioid and giant cells and appeared to be lost from caseous areas.

The observations recorded below are concerned with the content of *protein* as well as nucleic acid in caseous material.

MATERIALS AND METHODS

The tissues examined consisted of enlarged caseating lymph nodes taken by biopsy from 2 patients with chronic tuberculous lymphadenitis, another caseating node removed post mortem from a boy who died of generalized tuberculosis, and a caseous mass with surrounding fibrosis from a patient with chronic pulmonary tuberculosis.

After fixation (Susa®, formol saline followed by picro-sublimate, and formol saline alone were employed respectively as fixatives), the tissues were dehydrated in ethanol, cleared in xylene or chloroform, and embedded in wax. Sections 5 μ in thickness were cut. These were mounted on albuminized slides *except* for sections to be stained for protein. For the latter sections *no* adhesive was used, but firm attachment to the slides was secured by incubation overnight in an oven at 60°C.

After removal of wax with xylene followed by ethanol, the sections were washed in running tap water. In the case of tissue fixed in mercuric salts, treatment with iodine followed by thiosulfate was initially employed to remove any possible precipitates, but later it was found that washing with running tap water for twenty-five minutes sufficed for this purpose. The sections stained by pyronine were in no case treated with iodine, since oxidation might generate acidic groups and so affect the intensity of basophilia.

The sections were stained by the following methods:

Harris' hematoxylin and eosin.

Tetrazo: Protein was demonstrated by the tetrazo method of Danielli (5). This method depends on coupling one end of a tetrazotized molecule of benzidine to the phenolic (and certain other cyclic) groups in the tissue proteins with subsequent coupling of the other end to β-naphthol. In this way an intensely colored dyestuff is formed which is bound by a

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covalent linkage into the tissue proteins so as to form an integral part of their macromolecular structure. The sections were first cooled to 0–4°C. in 0.025 M borate buffer (pH = 9.0) and then stained by the tetrazo method of Danielli, employing the borate buffer technique of Dixon (6); the method followed was the same as that previously described (6, 7) except that the period of terminal coupling in β-naphthol buffer was increased from 0.25 hour to 0.5 hour. The sections were mounted finally in DPX mountant. After coupling with β-naphthol the previously yellow tetrazotized sections developed an intense orange-red color in regions rich in protein.

Tetrazo following benzoylation: Danielli (5) showed that prior treatment with benzoyl chloride in pyridine blocks the cyclic groups of proteins so that they can no longer be coupled with diazonium salts. Elimination of chromogenic capacity by previous benzoylation thus provides confirmation that proteins are indeed responsible for coloration of tissues treated by the tetrazo technique. Sections were washed in ethanol and then immersed in benzoyl chloride-pyridine mixture (1 volume:10 volumes) for 18 hours in the dark at room temperature. After washing with ethanol and running tap water, the sections were treated by the tetrazo technique as above.

Feulgen method: Desoxyribonucleic acid was demonstrated by the method of Feulgen and Rossenbeck; the following technique was used. After washing in three changes of distilled water the sections were heated for 8 minutes in a glass beaker containing N hydrochloric acid maintained at 60 to 63°C. They were then washed in distilled water and immersed in Schiff's reagent for 0.5 hour. After washing with three changes of aqueous sulfurous acid for more than 7 minutes, the sections were placed in running tap water for 5 minutes, dehydrated in ethanol, cleared in xylene, and mounted in DPX. Control sections not heated in N hydrochloric acid but otherwise treated by the same technique were always included. The Schiff's reagent and the sulfurous acid were made up according to McManus (8).

Pyronine: Pyronine *Y* or *G* colors both ribonucleic acid and desoxyribonucleic acid pink; pyronine is more intensely bound by ribonucleic acid than by desoxyribonucleic acid (9, 10). Other intracellular acidic substances also bind pyronine, so that this staining reaction is only indicative of the presence of nucleic acids. Rapid passage through ethanol removes the stain which is held by protein, but the nucleic acids are still colored, provided the period in ethanol is very short. The sections were immersed for 0.5 hour in a solution of the following composition: pyronine *G* "extra"[2] 5 gm., phenol 2.5 gm., ethanol 25 ml., glycerol 200 ml., made up to 1 liter with distilled water. After *very* rapid passage through tap water and ethanol successively, the sections were cleared in xylene and mounted in DPX neutral mountant.

Results

General picture of sections stained with hematoxylin and eosin: The lymph nodes from the patients with chronic tuberculous adenitis consisted of centrally situated confluent caseous material surrounded peripherally by discrete tubercles embedded in lymphoid tissue (figure 1). In the other node examined (from a patient with acute tuberculosis) the areas of caseation were less extensive.

Some of the tubercles in these nodes were in the early follicular stage and contained spherical and ovoidal masses of epithelioid cells and giant cells; in others the centers of the follicles were caseous and brightly eosinophilic, while the epithelioid cells and giant cells occurred at the periphery (figure 2). The epithelioid cells were both stellate and filiform; the latter possessed elongated processes which extended inwards toward the caseous centers of the tubercles. Nearly all basophilic material had vanished from the necrotic central regions of many of

[2] G. T. Gurr, Ltd.

FIG. 1. Segment of caseous gland. Hematoxylin and eosin stain (× 12)

the discrete tubercles; but some shrivelled pyknotic nuclei were visible around these areas of total karyolysis.

The central regions of the lymph nodes with confluent caseation were entirely necrotic and were formed by the fusion of discrete tubercles. The material derived from the original caseous centers of the fused tubercles was stained bright pink, but the necrotic lymphocytes were more faintly stained except for some purple granules formed by nuclear karyorrhexis.

In the specimen of pulmonary caseation examined, the loss of basophilia to hematoxylin was not so complete. Within the fibrous covering a wide zone of epithelioid cells surrounded the caseous mass. The innermost epithelioid cells contained elongated and shrivelled basophilic nuclei. The caseous region was situated inside this zone of pyknosis; it contained numerous shadowy nuclear remnants, which, although indistinct, were still faintly basophilic. Karyolysis was incomplete. In the inner parts of this specimen necrotic polymorphonuclear leukocytes were present in the degenerate caseous material.

Tetrazo: The caseous areas of the discrete tubercles were most intensely colored by the tetrazo method (figures 3, 4, 5, and 6). The areas of confluent necrosis in the middle of the caseous nodes were also well stained, but in this region the parts derived from the caseous centers of the original tubercles were richer in protein than the more faintly colored zones of necrotic lymphocytes.

The epithelioid cells and giant cells were strongly colored, but the central necrotic regions of the discrete tubercles contained even more abundant protein (figures 3, 4, 5, and 6). In figure 4 may be seen a highly magnified view of a giant cell and epithelioid cells adjoining a caseous area; all of these are illustrated at lower magnification in figure 6. The intense coloration of the caseous region is especially prominent.

In figures 3 and 5 may be seen the same areas stained by the tetrazo method

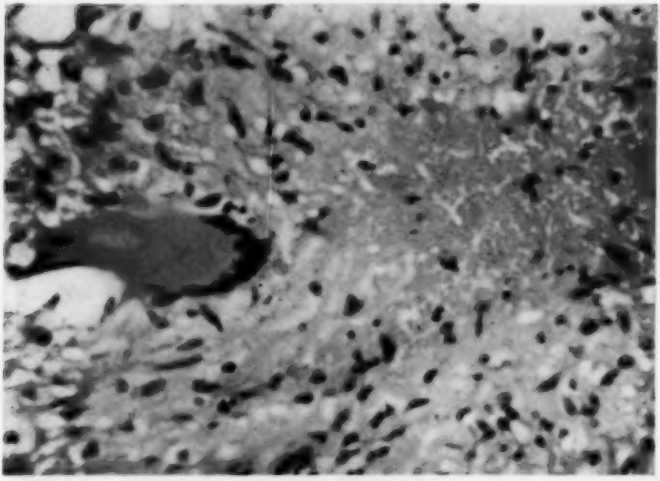

FIG. 2. Necrotic center of a tubercle. Hematoxylin and eosin stain (× 410)

as are illustrated respectively in figures 1 and 2 stained by hematoxylin and eosin. The intensely colored (orange-red) caseous areas in the sections stained by the tetrazo method correspond to the regions which are brightly eosinophilic after staining with hematoxylin and eosin. In figure 5 the copious protein is demonstrated in the filiform type of epithelioid cell as well as in the caseous center of the tubercles. Owing to the general intensity of coloration of the necrotic central elements, it is not possible to discern with certainty whether nuclear remnants contribute to the very substantial quantities of protein in the granular necrotic centers of the tubercles. However, the spherical appearance and size of some of these particles indicate that they may be nuclear in origin. Much of this tetrazo-positive debris is probably derived from the cytoplasm of the necrotic cells. The periphery of the pulmonary caseous mass is also rich in tetrazo-positive protein. The intensity of staining, however, diminishes centrally in this specimen, notably in the regions which contain necrotic polymorphonuclear leukocytes. This loss of protein may be caused by secondary digestion by the enzymes of the leukocytes.

Tetrazo following benzoylation: Along with the sections treated by the tetrazo method, other sections of each tissue, which had been benzoylated beforehand, were stained at the same time. The contrast of the latter with the unbenzoylated sections was striking; only a faint color was given by caseous material in the benzoylated controls, whereas the areas of caseation were intensely colored in the sections which had not received prior benzoylation.

In figure 7 the same tubercle may be seen which is illustrated in figures 2 and 5, but in this case from a section benzoylated before treatment by the tetrazo method. The caseous center of the tubercle is now almost uncolored, and the surrounding cells are only very faintly tinted.

These observations support the view that proteins containing cyclic groups,

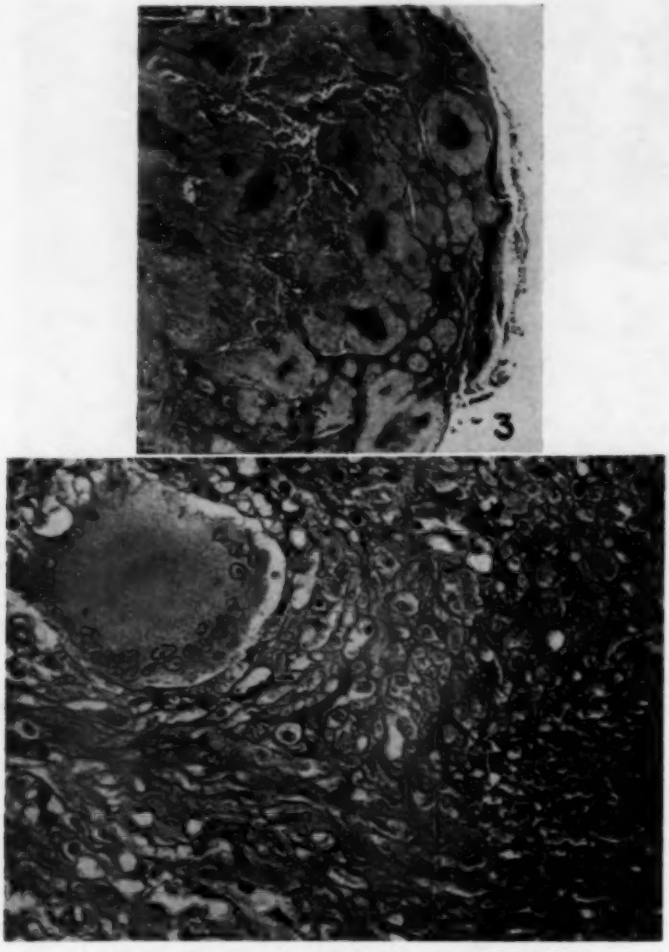

FIG. 3. Segment of caseous node. Tetrazo (× 12)

FIG. 4. Portion of a tubercle; giant cell, epithelioid cells, and caseous material (at bottom right). Tetrazo (× 420)

which are blocked by benzoylation, are indeed responsible for the intense coloration given by the tetrazo method.

Feulgen method: Both living and pyknotic nuclei in the tissues examined were well colored, while in the control sections not heated with hydrochloric acid only very faint coloration was visible.

Loss of nuclear material by karyolysis accounts for the absence of color in many of the areas of caseation (figures 8, 9, 10, and 11).

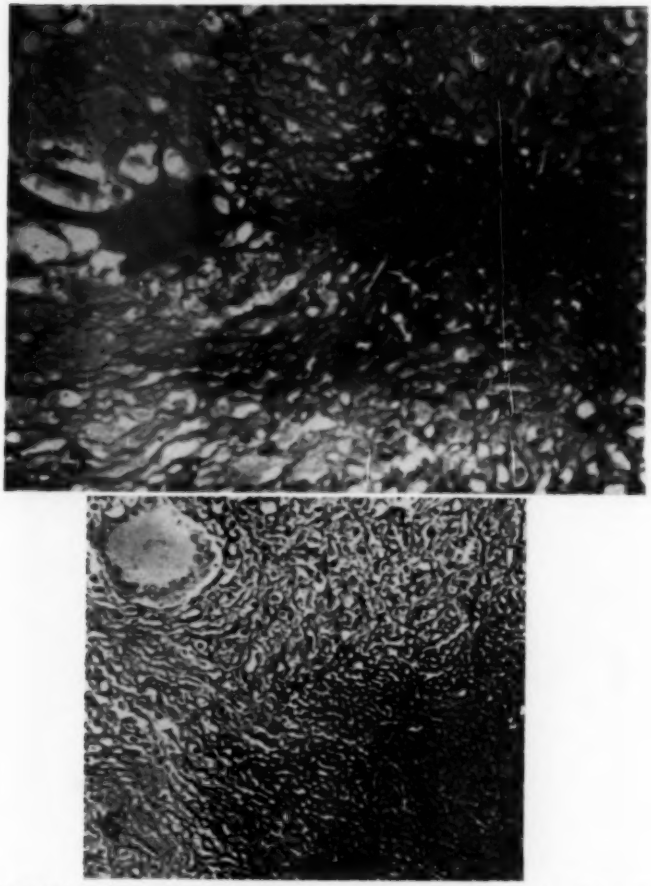

FIG. 5. Same tubercle as in figure 2; intense coloration of caseous matter. Tetrazo (× 420)
FIG. 6. Same tubercle as in figures 4 and 9. Caseous matter bottom right. Tetrazo (× 215)

In the lymph nodes the nuclei of the lymphocytes were especially prominent at the periphery of the tubercles and in the unaltered lymphoid tissue. The nuclei of the epithelioid cells were also well colored. As the total desoxyribonucleic acid per nucleus is constant in somatic cells (11) and since the nuclei to epithelioid cells are larger than in lymphocytes, it is not surprising that the lymphocytic nuclei were the more intensely colored. Near the caseous central regions of the tubercles some of the nuclei were shrivelled and pyknotic (figures 8, 9, and 11); their dense coloration is due to contraction in volume either to elongated (figures 8, 9) or spherical pyknotic remnants (figure 11). Presumably these intensely stained pyknotic nuclei still contained their full complement of desoxyribonucleic acid compressed into a smaller volume.

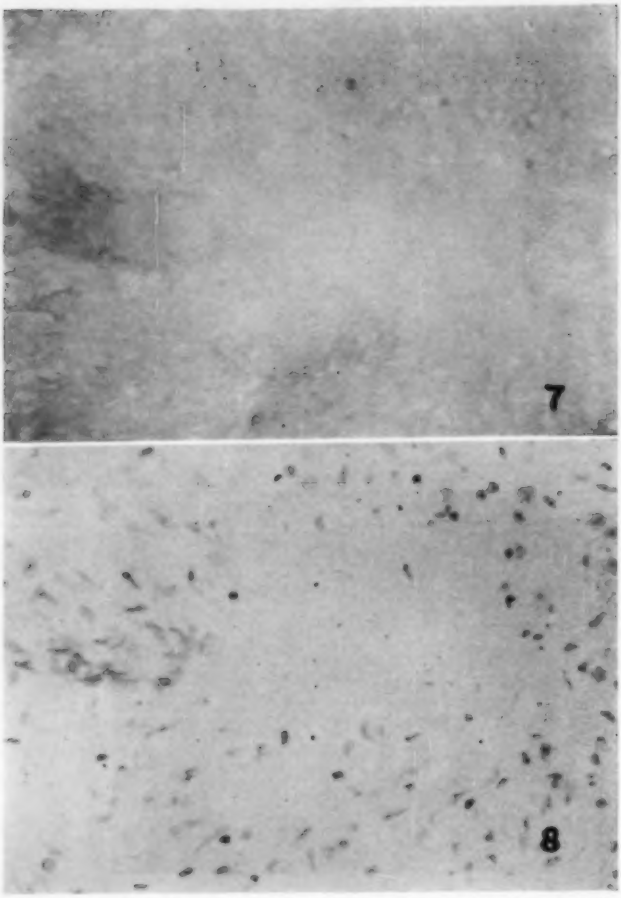

FIG. 7. Same tubercle as in figures 2 and 5. Tetrazo after previous benzoylation. (× 215)
FIG. 8. Same tubercle as in figures 2, 5, and 7; central karyolysis. Feulgen stain (× 410)

Sections stained by the Feulgen method may be seen in figures 8 and 9. The areas chosen come from the same tubercles shown in figures 5 and 6 stained by the tetrazo technique. It is clear from figures 8 and 9 that all desoxyribonucleic acid may be lost from areas of caseation, although so much protein is retained. The granular caseous debris in areas of confluent caseation is also frequently devoid of Feulgen-positive remnants (figure 10).

Along the margins of caseous areas the sequence of nuclear changes from without often appears as a uniformly progressive process. From the large faintly colored young epithelioid cells through the stage of shrivelled pyknosis inwards to the zone of terminal karyolysis with complete disappearance of desoxyribonu-

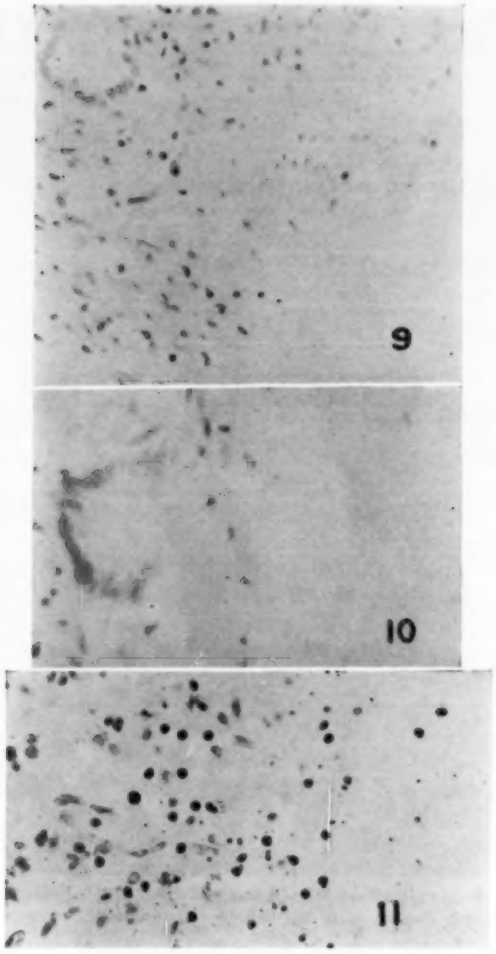

FIG. 9. Same tubercle as in figure 4; caseous matter bottom right. Feulgen stain (× 260)

FIG. 10. Giant cell and adjoining area of caseation; complete karyolysis. Feulgen stain (× 420)

FIG. 11. Pyknosis and karyolysis of epithelioid cells adjoining caseous area. Progressive process from left to right. Feulgen stain (× 440)

cleic acid, there is apparently a constant pattern of nuclear degeneration (figure 11).

In the specimens of pulmonary caseous tissue examined, karyolysis was not so complete. The epithelioid cells indeed showed uniform pyknosis to shrivelled remnants rich in desoxyribonucleic acid (figure 12). Further dissolution was

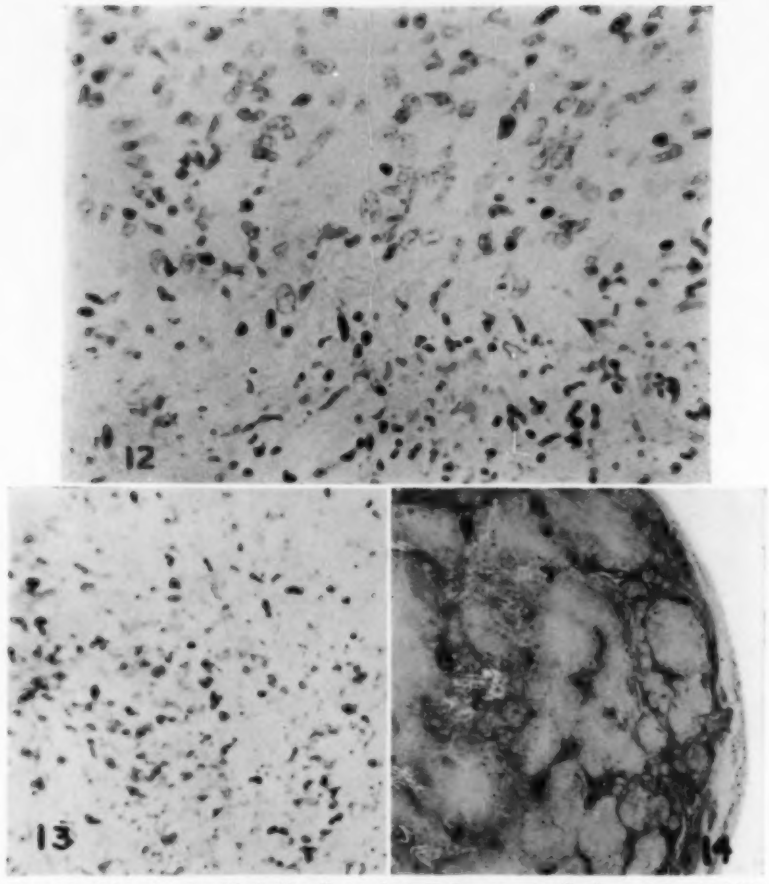

FIG. 12. Pyknosis of epithelioid cells to shrivelled remnants. Feulgen stain (× 440)

FIG. 13. Incomplete karyolysis of epithelioid cells; nuclear fragments. Feulgen stain (× 440)

FIG. 14. Segment of a caseous gland. Pyronine *G* (× 12)

incomplete and the deeper parts of the caseous mass retained shadowy Feulgen-positive nuclear debris (figure 13), including the fragments of disrupted nuclei (karyorrhexis).

Pyronine: The prominent feature of the caseous lymph nodes in sections stained by pyronine is the almost complete absence of color from the areas of caseation (figure 14). The cytoplasm and nuclei of the lymphocytes were well colored. The cytoplasm of the epithelioid cells and giant cells was only a faint pink, but their nuclear membranes and chromatin were colored more definitely. In the giant cells the cytoplasmic basophilia was mainly peripheral, and the central portions

of the cells were frequently devoid of color. Some pink shrivelled pyknotic nuclei adjoined the areas of caseation, but the caseous necrotic centers of the tubercles were entirely untinted by pyronine; these latter regions were thus depleted of ribonucleic acid as well as of desoxyribonucleic acid.

Discussion

The present observations establish that caseous material contains abundant protein. Caseation usually involves the disappearance of desoxyribonucleic acid (stainable by the Feulgen method) from the nuclei of the dead cells. This change is the essence of karyolysis. The capacity to stain with pyronine (probably due to ribonucleic acid) is simultaneously lost from the cytoplasm. Protein stained by the tetrazo method is, however, preserved and concentrated in the necrotic tissue.

Nucleic acids: Loss of desoxyribonucleic acid from the nuclei of necrotic cells in tissue transplants was recorded by Leuchtenberger (12). She found that pyknosis and karyolysis may be accompanied by the loss of both protein and desoxyribonucleic acid from the nuclei; in early pyknosis the loss of desoxyribonucleic acid was small, but this became substantial later in pyknosis. The terminal stage of pyknosis with loss of chromatin, however, merges into karyolysis, and, since *dissolution* of nuclear material is occurring simultaneously, this change should be regarded strictly as *karyolytic*. In cloudy swelling of renal tubules, caused by the injection of bacterial toxins, Fonnesu and Severi (13) noted loss of desoxyribonucleic acid from the nuclei of the injured cells. In caseous necrosis of tuberculosis, Monaci and Coppitz (4) recorded lack of desoxyribonucleic acid except for some nuclear remnants; but they also described scarcity of this acid in the epithelioid cells in tuberculosis. From the present investigation it is apparent that the nuclei of young epithelioid cells in tubercles contain considerable quantities of desoxyribonucleic acid; this is not confined to so small a space as in the smaller nuclei of lymphocytes and thus appears less intensely stained.

The present study shows that the nuclei of the epithelioid cells in caseating lymph nodes first become pyknotic and later disappear. The intermediate increase of intensity of the Feulgen reaction in pyknotic nuclei is associated with substantial diminution in nuclear volume and concomitant increase in the concentration of stainable desoxyribonucleic acid in each shrivelled pyknotic nucleus (figures 9, 11, and 12). Human somatic nuclei during interphase usually contain a constant amount of desoxyribonucleic acid (11). But, just as the minute nuclei of lymphocytes appear more strongly colored by the Feulgen method than the larger nuclei of epithelioid cells, so also the shrivelled pyknotic nuclei are more intensely stained than their living precursors (figures 11, 12).

Later the pyknotic nuclei disappear so that the central regions of the tubercles are devoid of desoxyribonucleic acid (figures 8, 9, 10, and 11). This process constitutes karyolysis. The transition of the nuclei of epithelioid cells through the successive stages of pyknosis and karyolysis is often a progressive process so that the margins of caseous areas may show a uniform sequence in this synchronous pattern of degeneration. Sometimes, however, little intermediate pyknosis is

visible and karyolysis or nuclear fragmentation (karyorrhexis) are the first changes observed.

In the tuberculous lymph nodes examined, the terminal karyolysis of caseation generally involves total loss of stainable desoxyribonucleic acid (figures 8, 9, 10, and 11). In the specimen of pulmonary caseous tissue studied, however, many shadowy nuclear remnants and granular fragments, containing appreciable amounts of Feulgen-positive material, persist even in the deeper necrotic zone (figure 13); here karyolysis is only partial.

The loss of capacity to stain with pyronine found in the caseous areas of the lymph nodes (figure 14) is in accordance with the findings of Monaci and Coppitz (4), who noted at least partial loss of ribonucleic acid from caseous material and from the centers of tuberculous giant cells. Loss of ribonucleic acid from the cytoplasm of necrotic cells is one of the earliest sequels of cellular injury (14, 2, 3).

The frequent persistence of protein in dead cells is well established. Corper (1) found that protein is retained in ischemic necrosis of the spleen long after cell nuclei have disappeared. Dixon (2) observed persistence of protein (stained by the tetrazo method) in the cell bodies of necrotic neurones after the total loss of cytoplasmic ribonucleic acid (stainable by pyronine); protein is similarly retained in the cells of infarcts in the spleen and kidney. It was suggested (2) that rapid death of the cells can destroy their capacity to absorb water which may be essential for the autolysis of protein. Thus proteolysis is interrupted and the proteolytic enzymes of the dead cells eventually become inactive. Dixon and McCullagh (3) noted similar persistence of protein in liver cells killed by carbon tetrachloride, although cytoplasmic ribonucleic acid vanishes from the dying cells.

In a histochemical investigation of tuberculous lesions in animals, Grogg and Pearse (15) showed that the necrotic centers of tubercles in guinea pigs are rich in acid phosphatase and also contain alkaline phosphatase; it was concluded that these cell-free enzymes arise by disruption of polymorphonuclear leukocytes which previously contained them. The occurrence of enzymes would suggest that some protein is retained in caseous material. The presence of these active hydrolytic enzymes in caseous regions may mean that proteolytic enzymes can remain active in the necrotic centers of the tubercles. However, the results now reported with the tetrazo method indicate that either the proteases are inactive, or else the abundant protein is so altered in nature that it can no longer be degraded easily.

Marshall (16) studied tuberculous lesions by impregnation with silver. He found that argyrophil ("metalophil") epithelioid cells send long processes, which are invisible in ordinarily stained sections, into the centers of the tubercles. Argyophilia of this kind may depend on the presence of histidine in the cellular proteins (17). The tetrazo method demonstrates similar elongated (filiform) epithelioid cells which contain copious protein (figure 5).

The present observations show that caseous material is rich in substances capable of coupling with tetrazotized benzidine, which may subsequently be coupled with β-naphthol to form intensely colored dyestuffs. Moreover the generation of these colored compounds is inhibited by previous benzoylation.

Such characteristics of a tissue depend on the presence of the amino acids tyrosine, tryptophane, and histidine in the tissue proteins (5). It is thus apparent that caseous material is rich in protein.

Much of the caseous material is also brightly eosinophilic. This feature is in part due to the absence of desoxyribonucleic acid and ribonucleic acid and consequent lack of basophilia to hematoxylin, but it also depends on the retention and concentration of eosinophilic protein. The same regions, which show the strongest reactions for protein (figure 5), are also prominent for their intense eosinophilia (figure 2). No basophilia to pyronine remains in these areas owing to disappearance of desoxyribonucleic acid and ribonucleic acid. Feulgen-positive nuclear material has also vanished from these necrotic regions (figures 8, 9) which at the same time are so conspicuously rich in protein (figures 5, 6).

In the granular necrotic elements of caseous tubercles, which are so intensely stained by the tetrazo method, some of the remnants resemble nuclei in size and shape. It is possible that some nuclear protein remains in caseous material, although all desoxyribonucleic acid detectable by the Feulgen method has disappeared. Much of the necrotic elements in caseous material, however, are probably composed of cytoplasmic debris. These mummified cellular remnants still contain abundant protein.

The macromolecular fabric of living cells is in a state of dynamic equilibrium. Both protein and nucleic acid are continuously being fabricated to make good their constant katabolism and degradation. It is believed that ribonucleic acid is the template on which all cytoplasmic protein is manufactured and that desoxyribonucleic acid is responsible for the synthesis of genic protein as well as for the resynthesis of ribonucleic acid (18, 19). It is thus apparent that, although so much protein is present in the caseous cells, the lack of both desoxyribonucleic acid and ribonucleic acid entails the permanent cessation of the synthesis of cellular proteins. Caseous elements have thus passed the point in cellular dissolution beyond which there is no conceivable return; their disintegration, although only partial, is irreparable.

Summary

Caseous material in tuberculosis is conspicuously rich in protein stainable by the tetrazo method of Danielli. Desoxyribonucleic acid demonstrated by the Feulgen technique is lost in the terminal karyolysis of caseation. Cytoplasmic basophilia, probably due to ribonucleic acid, also disappears in caseous necrosis. The intense eosinophilia, frequently visible in caseous matter, is related to the retention and concentration of protein in the necrotic remnants as well as to the loss of nucleic acids which can be stained by basic dyes.

Sumario

La Proteína y el Acido Nucleico en la Necrosis Caseosa

Las materias caseosas en la tuberculosis son notablemente ricas en proteína colorable con la técnica del tetrazo de Danielli. El ácido deoxirribonucleíco descubrible con la técnica de Feulgen se pierde en la cariólisis terminal de la caseación. La basofilia citoplásmica,

debida probablemente al ácido ribonucleíco, también desaparece en la necrosis caseosa. La intensa eosinofilia, visible frecuentemente en la materia caseosa, se relaciona con la retención y concentración de proteína en los restos necróticos así como con la pérdida de ácidos nucleícos que puedan teñirse con los colorantes básicos.

Resume

Protéine et acide nucléique dans la nécrose caséeuse

Dans la tuberculose, la matière caséeuse est particulièrement riche en protéine colorable par la méthode au tétrazo de Danielli. L'acide désoxyribonucléique identifié par la technique de Feulgen disparaît dans la caryolyse terminale de la caséation. La basophilie cytoplasmique, probablement due à l'acide ribonucléique, disparaît également dans la nécrose caséeuse. L'éosinophilie intense, fréquemment visible dans la matière caséeuse, est en relation avec la rétention et la concentration de la protéine dans les tissus nécrotiques, ainsi qu'à la perte des acides nucléiques peuvant être colorés par les colorants basiques.

Acknowledgment

The writer is grateful to S. W. Patman for the photomicrography, and to P. J. Hart and W. A. Mowlam for valuable technical help.

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SERUM PROTEIN CHANGES IN EXPERIMENTAL TUBERCULOSIS[1]

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(Received for publication July 1, 1957)

INTRODUCTION

Serum protein changes, notably elevation of the gamma globulin and mucoprotein fractions (1–5), are associated with human tuberculosis. Similar changes have been noted in experimental tuberculosis in guinea pigs (6, 7), and further experimental studies indicate better understanding of the changes in tuberculosis in man. Therefore, the serum protein changes in various modifications of experimental tuberculosis, including inactive forms, were investigated by both electrophoretic and simple turbidimetric methods. The latter were included primarily to find out whether such simple methods would be helpful in the evaluation of guinea pig tuberculosis.

The biochemical alterations were correlated with the histologic picture, specific emphasis being laid upon the alterations of cytoplasmic basophilia. These alterations, demonstrated by pyroninophilia and identified by specific digestion with ribonuclease, are caused to the greatest extent in the liver by pentose nucleic acids, which have been related to protein synthesis or at least are considered to indicate the site of protein formation (8). It appeared worth while, therefore, to correlate abnormal increases in cytoplasmic basophilia in various cells with the increase of serum gamma globulin and the stage of the tuberculous infection.

MATERIALS AND METHODS

The material consists of 213 guinea pigs divided into several groups.

Nontuberculous Control Groups

Group 1a: 12 normal guinea pigs sacrificed at approximately 5 months of age.

Group 1b: 20 normal guinea pigs sacrificed between the ages of 7 and 20 months.

Group 2: 9 guinea pigs more than 10 months old which had abnormalities of nontuberculous origin when sacrificed; for instance, enlarged cervical lymph nodes.

Group 3a: 10 guinea pigs less than 6 months of age, infected once with 10^{-3} ml. of a 24-hour culture of Friedländer's bacilli in 1 ml. of saline solution and sacrificed 3 weeks later.

Group 3b: 11 guinea pigs more than 7 months old treated as those in *Group 3a*.

Group 4a: 13 guinea pigs less than 6 months of age, to which 0.03 ml. of carbon tetrachloride in 0.3 ml. of liquid petrolatum per 100 gm. of body weight was administered twice a week for 3 weeks; the animals were sacrificed 2 days after the last dose of carbon tetrachloride.

Group 4b: 5 guinea pigs more than 7 months old treated as were those in *Group 4a*.

Guinea Pigs Infected with Tubercle Bacilli

Guinea pigs of *Group 5* through *14* were infected subcutaneously in the right inguinal region with 0.1 mg. of H37Rv tubercle bacilli.

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[1] From the Municipal Tuberculosis Sanitarium, Chicago, Illinois and the Hektoen Institute for Medical Research of the Cook County Hospital, Chicago.

Animals of these groups (except those in *Group 5a*) were older than 7 months.

Group 5a: 25 guinea pigs sacrificed between 61 and 99 days after their tuberculous inoculation.

Group 5b: 16 guinea pigs sacrificed between 100 and 551 days after their tuberculous inoculation.

Group 6: 22 guinea pigs which received 20 mg. of isoniazid daily in their diet (9), starting 7 days after the inoculation with H37Rv and continuing for 60 days. These animals were sacrificed between 132 and 551 days after they were inoculated with tubercle bacilli, and no evidence of tuberculosis was found on necropsy.

Group 7: 8 guinea pigs treated as those in *Group 6* but sacrificed between 231 and 551 days; evidence of tuberculosis was found on necropsy.

Group 8: 12 guinea pigs infected and treated as those in *Group 6* except that 52 days after treatment was discontinued the animals were reinoculated with 0.1 mg. of H37Rv tubercle bacilli and were sacrificed between 222 and 479 days; evidence of tuberculosis was found on necropsy.

Group 9: 8 guinea pigs infected and treated as those in *Group 6*; 2 months after treatment was discontinued they were fed a synthetic diet containing 0.3 per cent of ethionine for 3 weeks and were then sacrificed; no evidence of tuberculosis was found on necropsy. The liver revealed moderate hepatocellular degeneration, single cell necrosis and regeneration, as well as interstitial proliferating ductular cells and inflammatory infiltrations.

Group 10: 1 guinea pig, identical with those in *Group 9* except that evidence of tuberculosis was found on necropsy.

Group 11: 5 guinea pigs, similar to those in *Group 6*; 4 months after treatment was discontinued carbon tetrachloride was administered as in *Group 4a*; no evidence of tuberculosis was found on necropsy. The liver revealed centrolobular necrosis with intermediate fatty metamorphosis and portal and periportal inflammation.

Group 12: 2 guinea pigs identical with those in *Group 11* except that evidence of tuberculosis was found on necropsy.

Group 13: 11 guinea pigs infected and treated as those in *Group 6*; 52 days after treatment was discontinued they were inoculated with Friedländer's bacilli as were those in *Group 3a*; 3 weeks later no evidence of tuberculosis was found on necropsy.

Group 14: 5 guinea pigs inoculated with H37Rv tubercle bacilli as were those in *Group 1*; 7 months later they were inoculated with Friedländer's bacilli as were the animals in *Group 3a*. Evidence of tuberculosis was found on necropsy.

Guinea Pigs Vaccinated with BCG

All animals in *Groups 15* and *16* were more than 7 months old.

Group 15: 12 guinea pigs inoculated with 1 mg. of BCG and sacrificed 5 months later.

Group 16: 6 guinea pigs vaccinated with BCG, inoculated with Friedländer's bacilli 4 months later, and sacrificed 3 weeks after the latter inoculation.

Animals which died spontaneously were not included in this study. A thorough autopsy was performed in sacrificed animals, and liver, lung, and spleen were subjected to routine histologic study. Moreover, two sections each of Carnoy fixed material, one of which had been digested previously with ribonuclease, were stained with methyl-green-pyronine by the method of Kurnick (10) which was modified by using a chloroform-extracted solution of pyronine *B* instead of pyronine *Y*. The degree of pyroninophilia removable by ribonuclease in various cells was graded 0 to 3-plus without knowledge of the experimental history and the anatomic findings.

Serum was obtained from heart blood, and total protein was determined by micro-Kjeldahl and Nesslerization. Gamma globulin was determined turbidimetrically (11) according to Huerga and Popper. Using this method, there is a linear relationship between the optical densities and gamma globulin concentration from 3 down to 0.8 gm. per 100 ml. Below 0.8 gm. per 100 ml. a different relationship was found between turbidimetric readings

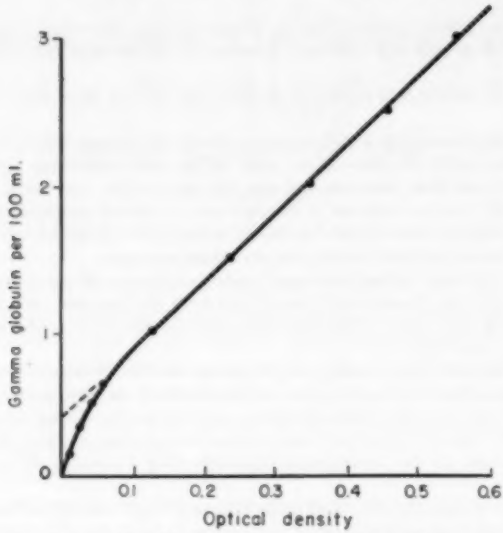

FIG. 1. Graph showing relationship between concentration of gamma globulin in serum and optical density. For values below 0.8 gm. per 100 ml. this relationship was determined by diluting normal serum with saline so as to make a concentration of gamma globulin from 1.0 gm. down to 0.2 gm. per 100 ml.

and concentration. This relationship can easily be determined by diluting normal serum with saline so as to make a concentration of gamma globulin from 1 gm. down to 0.2 gm. per 100 ml. This correlation is shown in figure 1. The instrument used in these experiments was the Coleman Universal spectrophotometer, with a 19 mm. round cuvette. If using other instruments with lower response to turbidity, or working with serums of low gamma globulin concentration, a wave length of 500 mu. will result in a greater degree of precision. Mucoproteins were determined turbidimetrically according to Huerga and associates (12) and, finally, zone electrophoresis was performed with quantitation of the bromphenol blue color (13) of the paper strips with a photometric scanner (Spinco Analytrol[2]). The serum protein fractions were expressed in gm. per 100 ml., and these absolute amounts were calculated with total protein as reference.

RESULTS

Nontuberculous Guinea Pigs

Total proteins were significantly higher in normal guinea pigs more than 7 months old (*Group 1b*) than in younger animals (*Group 1a*) (table 1). This increase was almost equally distributed between all of the serum protein fractions and resulted in an average increase in gamma globulin of about 0.2 to 0.3 gm. per 100 ml. in both chemical and electrophoretic determinations. The difference in the mucoprotein concentration was not significant.

In the presence of nontuberculous abnormalities, of which the enlargement of the cervical lymph nodes was the most conspicuous (*Group 2*), the serum gamma globulin was significantly elevated, as compared with the values for the older

animals (*Group 1b*), as indicated by both the turbidimetric and the electrophoretic measurements. Similarly serum mucoproteins were moderately elevated and the albumin was depressed; nevertheless, the total proteins were higher. Cytoplasmic basophilia of Kupffer and other mesenchymal cells was not significantly altered.

Injection of Friedländer's bacilli failed to produce any significant change in younger animals with the exception of an increase of alpha globulin concentration (*Group 3a*) while, in older animals (*Group 3b*), there was an increase in mucoprotein and a slight reduction in albumin concentrations.

In old and young guinea pigs intoxicated with carbon tetrachloride (*Groups 4a* and *4b*), a slight increase in the gamma globulin and a reduction of the mucoprotein were associated with an increase of the basophilia of the Kupffer cells, but not with any other mesenchymal reaction (table 2). The total proteins were lower in the older animals (*Group 4b*).

Guinea Pigs Inoculated with Tubercle Bacilli

Inoculation with virulent tubercle bacilli produced in both older and younger guinea pigs (*Groups 5a* and *5b*) a marked rise within 99 days in gamma globulin, as measured turbidimetrically and electrophoretically. In addition albumin was reduced and alpha globulin elevated. The mucoprotein concentrations rose in prolonged tuberculosis. In both groups the total proteins were considerably higher than normal, compared with their respective age controls. In both instances a very marked pyroninophilia of the Kupffer cells and of the mesenchymal cells was seen in granulomas and lung and spleen (table 2).

Animals with latent tuberculosis due to treatment (*Group 6*) revealed no definite increase in gamma globulin or any other significant alteration in serum protein, except for a slight rise in mucoprotein, when compared to the older control animals. In the instances in which tuberculosis developed despite treatment (*Group 7*), the alpha and gamma globulins as well as the serum mucoprotein were significantly elevated and the albumin was reduced. This was associated with a far more marked elevation of the pyroninophilia of the Kupffer cells as well as of the other mesenchymal elements. Infected animals treated with isoniazid and subsequently reinfected with tubercle bacilli (*Group 8*) had a very high concentration of serum gamma globulin, an increase in mucoprotein, and a drop in albumin. This, however, was not associated with as marked an elevation of pyroninophilia of the Kupffer cells as was evident in the previous instance.

Infected animals treated with isoniazid and then fed an ethionine-containing diet (*Group 9*) developed a moderate elevation of gamma globulin and an increase of mucoprotein as well as a relatively high serum albumin, resulting in a higher concentration of total protein despite the drop of the beta and alpha globulin concentrations. In the one animal in which obvious tuberculosis developed (*Group 10*), the gamma globulin concentration was even higher. Similarly, animals successfully treated for tuberculosis but subsequently injected with carbon tetrachloride (*Group 11*) developed a moderate elevation of gamma globulin and mucoprotein, with a drop in beta globulin and albumin. When tuberculosis de-

TABLE 1

SERUM PROTEIN FRACTIONS (GM. PER 100 ML.) IN VARIOUS TYPES OF GUINEA PIG TUBERCULOSIS IN COMPARISON TO NONSPECIFIC INFECTIONS AND LIVER INJURY

Group Number	Experimental Group	Number of Animals	Chemical Determinations						Electrophoretic Determinations							
			Total Proteins (Mean)	SEm/(OD/SE-diff.*)	Muco Proteins (Mean)	SEm/(OD/SE-diff.*)	Gamma Globulin (Mean)	SEm/(OD/SE-diff.*)	Gamma Globulin (Mean)	SEm/(OD/SE-diff.*)	Beta Globulin (Mean)	SEm/(OD/SE-diff.*)	Alpha Globulin (Mean)	SEm/(OD/SE-diff.*)	Albumin (Mean)	SEm/(OD/SE-diff.*)
1a	Young normals (approx. 5 months)	12	5.2	0.11/—	0.314	0.013/—	0.69	0.04/—	0.8	0.06/—	0.7	0.05/—	1.2	0.05/—	2.8	0.1/—
1b	Older normals (7–20 months)	20	6.2	0.11/(6.4)†	0.324	0.017/(.5)†	0.92	0.04/(4.0)†	0.8	0.08/(3.0)†	0.9	0.05/(2.8)†	1.5	0.06/(3.7)†	3.0	0.1/(1.4)†
2	Cervical nodes	9	6.7	0.05/4.0	0.358	0.038/.8	1.42	0.1/4.5	1.6	0.01/8.4	1.1	0.08/2.2	1.6	0.06/1.2	2.6	0.08/3.0
3a	Friedländer's infection; young	10	5.8	0.28/2.0	0.360	0.042/1.1	0.82	0.09/1.3	0.8	0.07/1.1	0.8	0.09/1.0	1.5	0.04/5.0	2.6	0.08/1.5
3b	Friedländer's infection; old	11	5.9	0.13/1.9	0.508	0.018/7.0	0.95	0.03/0.4	0.8	0.03/0.0	1.1	0.10/1.8	1.5	0.03/0.0	2.6	0.15/2.2
4a	Carbon tetrachloride; young	13	5.3	0.24/0.5	0.230	0.022/3.2	0.91	0.16/1.3	0.7	0.04/2.9	0.7	0.04/0	1.3	0.13/0.7	2.4	0.18/1.9
4b	Carbon tetrachloride; old	5	5.5	0.07/5.6	0.189	0.013/6.4	1.04	0.15/.8	—	—/—	—	—/—	—	—/—	—	—/—
5a	Tuberculous controls (61–99 days)	25	6.0	0.15/4.4	0.254	0.16/2.5	1.55	0.11/7.1	1.7	0.21/5.5	0.9	0.06/2.5	1.5	0.15/1.9	2.1	0.09/5.4
5b	Tuberculous controls (100–551 days)	16	6.8	0.16/3.1	0.500	0.030/5.1	1.61	0.15/4.3	1.9	0.21/4.8	1.1	0.08/2.5	1.8	0.11/2.5	2.1	0.06/7.5
6	Tuberculous, isoniazid-treated (negative for tubercle bacilli)	22	5.9	0.10/1.5	0.371	0.019/2.1	0.94	0.05/.3	0.8	0.20/0.0	0.8	0.03/1.6	1.5	0.06/0.0	2.9	0.09/0.8
7	Tuberculous, isoniazid-treated (positive for tubercle bacilli)	8	6.6	0.14/2.5	0.498	0.057/2.9	1.41	0.07/6.1	1.7	0.11/6.4	0.8	0.05/1.0	1.8	0.11/2.5	2.4	0.21/2.6
8	Tuberculous, isoniazid-treated, reinfected (positive for tubercle bacilli)	12	6.7	0.20/2.5	0.434	0.029/3.3	1.52	0.16/3.5	1.9	0.26/3.7	1.0	0.10/0.9	1.6	0.08/1.1	2.6	0.21/1.6
9	Tuberculous, isoniazid and ethionine (negative for tubercle bacilli)	8	6.4	0.35/0.6	0.482	0.53/2.8	1.20	0.15/1.8	1.0	0.15/1.2	0.5	0.06/5.0	1.3	0.08/2.0	3.6	0.37/1.6

10	Tuberculous, isoniazid and ethionine (positive for tubercle bacilli)	1	6.5	—/—	0.382	—/—	1.80	—/—	1.5	—/—	0.6	—/—	1.2	—/—	3.2	—/—
11	Tuberculous, isoniazid and CCl_4 (negative for tubercle bacilli)	5	6.1	0.08/0.8	0.441	0.069/1.6	1.20	0.06/1.0	1.4	0.23/2.5	0.7	0.07/2.5	1.4	0.09/1.0	2.6	0.40/1.0
12	Tuberculous, isoniazid and CCl_4 (positive for tubercle bacilli)	2	6.6	—/—	0.727	—/—	1.80	—/—	1.9	—/—	0.8	—/—	1.5	—/—	2.4	—/—
13	Tuberculous, isoniazid; Friedländer's infection (negative for tubercle bacilli)	11	5.6	0.12/4.4	0.395	0.028/2.2	0.85	0.05/1.1	0.9	0.10/0.8	0.8	0.05/1.4	1.3	0.04/2.8	2.6	0.09/3.0
14	Tuberculous and Friedländer's infection (positive for tubercle bacilli)	5	7.1	0.23/3.8	0.461	0.041/3.1	2.07	0.23/4.8	2.4	0.08/14.5	0.8	0.08/1.0	1.6	0.08/1.1	2.3	0.34/2.9
15	BCG	12	6.2	0.22/0.0	0.381	0.047/1.1	1.12	0.07/2.5	1.0	0.11/1.5	0.8	0.07/1.2	1.5	0.11/0.0	2.6	0.18/2.0
16	BCG, Friedländer's infection	6	5.6	0.10/3.5	0.381	0.022/2.5	0.83	0.07/1.1	0.8	0.20/0.0	0.8	0.09/1.0	1.5	0.08/0.0	2.6	0.08/3.0

* SE-Diff equals $\sqrt{SE_{m1}^2 + SE_{m2}^2}$: SE_{m1} and SE_{m2} represents the standard errors of the two measures whose difference is being studied.

† Values obtained when the older normals (*Group 1b*) are compared with the young normals (*Group 1a*). Each of the subsequent groups is compared with either *Group 1a* or *Group 1b* according to which is the comparable age group.

veloped (*Group 12*), the gamma globulin and the mucoprotein concentrations were markedly higher. Administration of Friedländer's bacilli to treated animals (*Group 13*) led to a slight decrease in albumin and an increase of mucoprotein, while nontreated tuberculous animals injected with Friedländer's bacilli (*Group 14*) showed marked rise of the gamma globulin, alpha globulin, and mucoprotein fractions, and a drop of the albumin concentration. Although the drop in albumin was marked in this group, the great increase in gamma globulin and in alpha globulin brought the total proteins to the highest concentration observed in these studies. The cytoplasmic basophilia was high in all sites.

TABLE 2

ESTIMATED AVERAGE OF CYTOPLASMIC PYRONINIA REACTION REMOVED BY RIBONUCLEASE IN VARIOUS SITES IN THE ANIMALS OF TABLE 1

Estimated Cytoplasmic Basophilia in	Groups																		
	1a	1b	2	3a	3b	4a	4b	5a	5b	6	7	8	9	10	11	12	13	14	15	16
Hepatic cells	1.2	1.2	1.2	1.8	1.1	1.0	1.9	1.1	1.3	1.4	1.2	1.1	1.1	1.0	1.0	1.0	1.5	1.5	1.0	1.5
Kupffer cells	0.5	0.5	0.4	0.5	0.5	0.9	1.1	2.0	2.3	0.6	1.9	1.3	0.8	1.0	0.9	1.5	0.8	2.3	0.5	2.0
Splenic cells	1.2	1.5	1.1	1.5	1.4	1.0	1.1	2.0	2.4	1.4	1.8	2.0	1.5	1.0	1.6	2.0	1.6	2.4	1.1	1.1
Granuloma cells	0.3	0.2	0.3	0.0	0.4	0.2	0.0	1.8	2.1	0.3	2.2	1.5	0.7	2.0	0.3	1.0	0.3	2.9	0.2	2.0

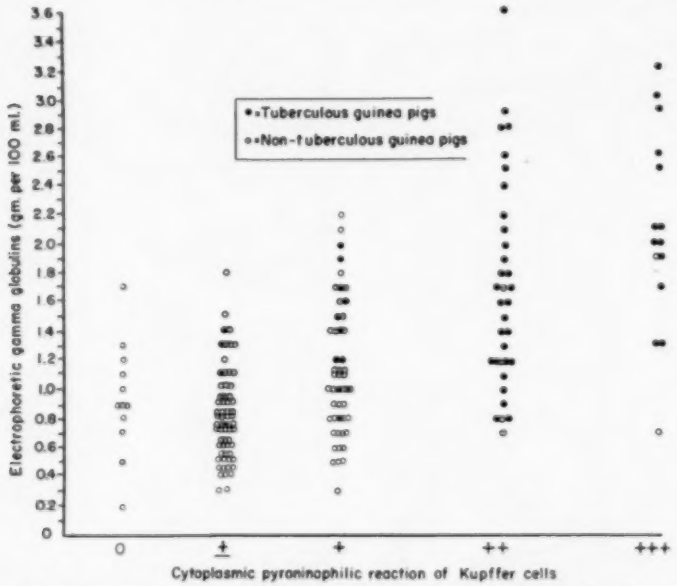

FIG. 2. Correlation between cytoplasmic pyroninophilic reaction of Kupffer cells with the serum gamma globulin concentration determined by electrophoresis.

BCG-Vaccinated Guinea Pigs

In animals immunized with BCG (*Group 15*) the serum gamma globulin rose slightly; the mucoproteins rose and the albumin dropped slightly. In animals treated with BCG and subsequently infected with Friedländer's bacilli (*Group 16*) there was a drop in the albumin concentration, and a marked basophilia of the Kupffer cells was observed.

Distribution of cytoplasmic basophilia: Cytoplasmic basophilia removed by specific digestion with ribonuclease was demonstrated in the tuberculous granulomas in guinea pigs in both the epithelioid cells and the histiocytes. In the epithelioid cells the cytoplasm showed a diffuse but not intensive pyroninophilia which was absent in the caseating areas. In contrast, in the smaller histiocytic cells, mainly on the periphery of the granulomas, the pyroninophilia was much stronger and was produced by closely packed, fine granules. In general the pyroninophilic reaction was more marked on the noncaseating periphery of the tuberculous lesion and therefore was more conspicuous in the smaller than in the

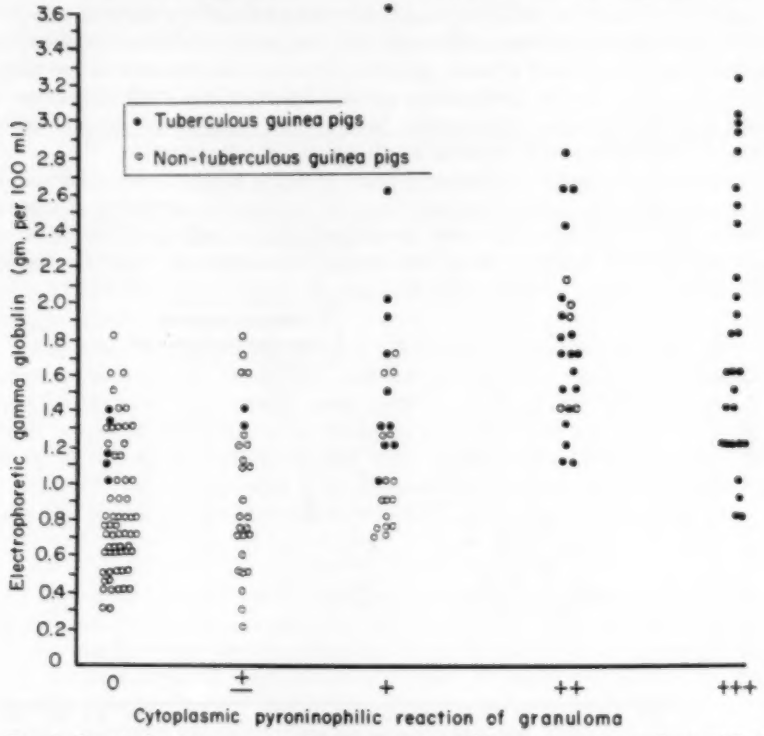

FIG. 3. Correlation between cytoplasmic pyroninophilic reaction of granuloma with the serum gamma globulin concentration determined by electrophoresis.

larger granulomas. Normally, only little pyroninophilic material was found in the lung, mainly on the periphery of the few lymph follicles. The normal spleen revealed pyroninophilic cells in fair number, especially on the periphery of the follicles, and less so in the reticuloendothelial cells of the white pulp. In the normal liver the cytoplasm of the liver cells contained large amounts of pyroninophilic material which decreased markedly in hepatocellular injury.

The Kupffer cells of the liver were normally free of cytoplasmic pyroninophilia, and positive reacting monocytes were hardly ever found in the portal tracts. In carbon tetrachloride and in ethionine intoxication, some Kupffer cells showed the pyroninophilic reaction, and around the centrolobular necrotic zone some of the accumulating histiocytes contained pyroninophilic material.

In tuberculosis, besides the reaction within and around the granulomas described above, the Kupffer cells revealed a diffuse increase of the pyroninophilia. In addition, in the portal tracts an accumulation of highly pyroninophilic wandering cells were found either scattered or in nodular forms. Similar nodules, composed of strongly pyroninophilic histiocytes, were found in the cytoplasm.

Attempts to correlate the cytoplasmic pyroninophilia of the Kupffer cells and of the cells of the tuberculous granuloma with the gamma globulin concentration (figures 2 and 3) revealed a rough parallel. However, the presence of too many abberations from the line designating parallel behavior was evidence against a direct, possibly causative, relationship between the cytoplasmic pyroninophilia of the studied cell elements and the gamma globulin concentration.

Relationship of elevation of gamma globulin to that of mucoproteins: Although in many experimental tuberculous conditions the serum concentration of gamma globulin and of mucoproteins rose simultaneously in individual instances, no statistical parallel between these two serum concentrations could be demon-

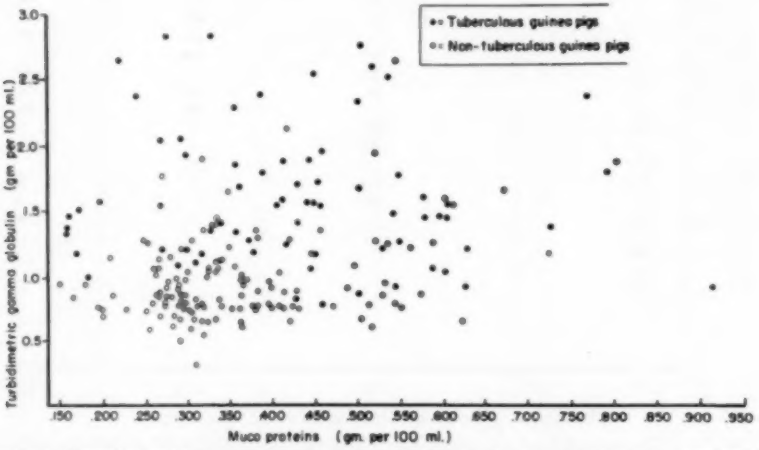
•• Tuberculous guinea pigs
○○ Non-tuberculous guinea pigs
Turbidimetric gamma globulin (gm per 100 ml.)
3.0
2.5
2.0
1.5
1.0
0.5
.150 .200 .250 .300 .350 .400 .450 .500 .550 .600 .650 .700 .750 .800 .850 .900 .950
Muco proteins (gm. per 100 ml.)

FIG. 4. Correlation between serum gamma globulin and mucoprotein concentrations in guinea pig tuberculosis.

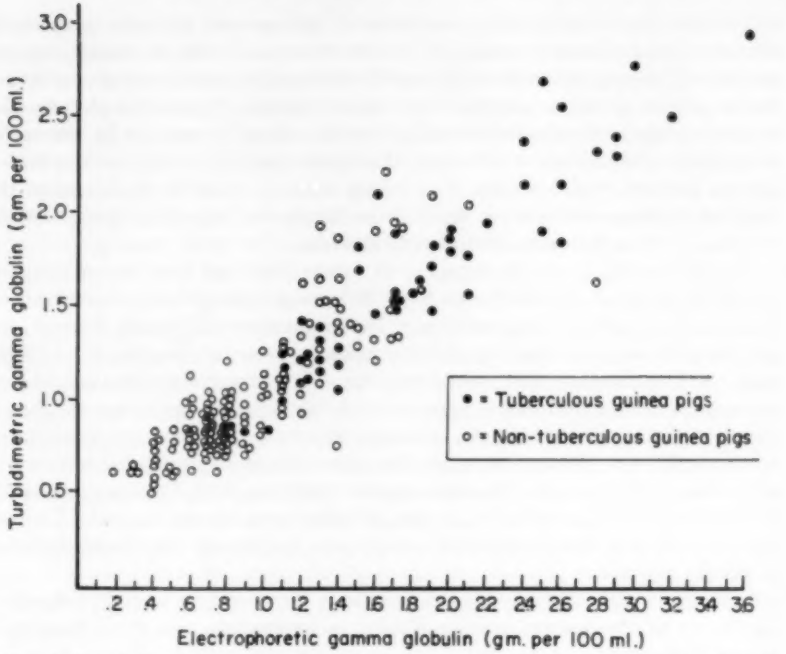
Turbidimetric gamma globulin (gm. per 100 ml.)
3.0
2.5
2.0
1.5
1.0
0.5
.2 .4 .6 .8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0 3.2 3.4 3.6
Electrophoretic gamma globulin (gm. per 100 ml.)
• = Tuberculous guinea pigs
○ = Non-tuberculous guinea pigs

FIG. 5. Correlation between gamma globulin concentrations determined electrophoretically and turbidimetrically in guinea pigs.

strated. Quite frequently one of the concentrations was very high while the other was in the normal range (figure 4).

Relationship between gamma globulin concentrations determined turbidimetrically and electrophoretically: In general the gamma globulin values determined by both methods showed a fairly good parallel, as indicated by the correlation value r = 0.857. There was a tendency for the values determined by zone electrophoresis to be distinctly higher with values of more than 1 gm. of gamma globulin per 100 ml. and lower with values of less than 0.8 gm. per 100 ml. than those determined by the turbidimetric method (figure 5).

DISCUSSION

Of the serum protein changes studied in experimental tuberculosis, the most regular and conspicuous appears to be the elevation of the gamma globulin, and this can be demonstrated just as easily by the simple turbidimetric method as by the more involved electrophoretic method, since the results by both methods are in close agreement. Similar elevations of gamma globulin, however, occur in guinea pigs with nontuberculous conditions, and this limits the diagnostic use of this procedure.

The mechanism of the gamma globulin elevation is just as conjectural in

tuberculosis as in many other conditions. If the gamma globulin elevation is related to an antibody formation, it is not associated with immunity because animals with latent tuberculosis (*Group 6*) which are resistant have no significant rise in gamma globulin concentrations. Quantitatively the gamma globulin rise is much too great in tuberculous animals to have been brought on by the excess of antibody globulin alone. The fact that older control animals have a higher gamma globulin concentration than young animals could be explained as the result of various sensitizations which occur during the longer life span, with the resultant increased formation of gamma globulin.

Animals in which the development of tuberculosis has been successfully inhibited by isoniazid administration have the same gamma globulin concentrations as control animals of comparable age. In the presence of hepatic damage, the gamma globulin concentration in these animals rises, but the rise is no higher than the liver damage alone would produce. With tuberculosis demonstrable at autopsy, a cumulative effect is apparent and this supports the viewpoint that in the successfully treated animals, although they have increased resistance to reinfection (7), no evidence exists for the alteration of an immunity response in the serum fractions. Also, the superimposed infection with Friedländer's bacilli did not lead to the elevation of gamma globulin levels in such animals. Vaccination with BCG however, produced a slight, yet statistically significant elevation of gamma globulin.

In animals with long-standing tuberculosis the infection with Friedländer's bacilli led to the highest gamma globlin concentration, clearly indicating a cumulative effect.

The site of the gamma globulin formation is not clearly established. In a previous investigation evidence was obtained that the excess gamma globulin in tuberculosis differs chemically from the preformed gamma globulin (7), and this raises the question of the site of formation of the excess gamma globulin. Elevation of gamma globulin as a rule runs parallel with the stainable cytoplasmic nucleoproteins in the cells of the granulomas as well as in the mesenchymal cells of the liver. This would suggest a common origin of gamma globulin from these cells. This would agree with a previously made assumption (14) that in liver injury the excess gamma globulin is formed in the Kupffer cells, while normally gamma globulin is produced by plasma cells (15), the reticuloendothelial cells of bone marrow (16), and lymph nodes (17). The parallel is not clear cut enough, however, to exclude the possibility that both gamma globulin formation and increased cytoplasmic pyroninophilia are independent reflections of the same cause, namely tuberculosis. It should be emphasized that in nonspecific infections, reflected in enlargement of the cervical lymph nodes, no elevation of the cytoplasmic basophilia is found despite high serum gamma globulin. Further investigations are required to establish whether the excess gamma globulin in guinea pig tuberculosis is formed in other sites than in nontuberculous animals.

Frequently, although not regularly reflecting the degree of the tuberculous process, the reduction of serum albumin is probably not the result of simple malnutrition since the total protein concentration is not reduced but is elevated.

There is no clear-cut relationship between the reduction of serum albumin and the cytoplasmic basophilia of the liver cells, which are generally accepted as the source of serum albumin. Therefore, no evidence exists that inability of liver cells to form albumin, possibly because of damage, accounts for the hypoalbuminemia. It is probable that the low albumin concentration is the result of preferential formation of gamma globulin from the available amino acids over the formation of albumin.

The variations of the mucoproteins, although not entirely parallel with that of gamma globulin, seem to be governed by similar processes.

Summary

In tuberculous guinea pigs the serum concentrations of gamma globulin and mucoproteins rise while the serum albumin drops. These changes, however, are not diagnostic, since they were found also in guinea pigs with nontuberculous abnormalities. There was no clear parallel between the elevation of mucoprotein and that of gamma globulin, the mucoprotein rising mainly in prolonged tuberculosis. Serum gamma globulin concentrations determined by chemical and electrophoretic methods agree well. The gamma globulin concentration in older guinea pigs used as controls is higher than in younger animals.

In tuberculosis rendered latent by isoniazid treatment, the serum concentration of gamma globulin is not elevated and the albumin concentration is not reduced. In animals inoculated with BCG, serum gamma globulin is slightly elevated and albumin slightly lowered. A superimposed hepatic injury or infection with Friedländer's bacilli did not alter the serum protein fractions in latent tuberculosis or in BCG-vaccinated animals beyond that of the effect of the hepatic injury per se, but in tuberculosis a cumulative effect was noted.

The cytoplasmic basophilia of the mesenchymal elements in the liver, of reticuloendothelial cells of the spleen, and of the epithelioid and lymphoid cells of the specific granulomas is very conspicuous in experimental tuberculosis. The parallel of the cytoplasmic basophilia of these mesenchymal elements with the gamma globulin rise is not clear enough to suggest strongly the localization of the excess gamma globulin formation into those elements. It is conceivable that the excess gamma globulin is made at the expense of albumin.

Acknowledgment

The writers wish to thank Dr. M. J. Schiffrin of Hoffmann-LaRoche, Inc., Nutley, New Jersey, for the isoniazid (Rimifon) used in these experiments. They also wish to thank Miss H. A. Dyniewicz, biochemist, Mr. Abdulla Ibraham, histologist, and Mr. George Hurubian, technician, for technical assistance in these studies.

Sumario

Las Alteraciones de la Proteína Sérica en la Tuberculosis Experimental

En los cobayos tuberculosos, se eleva la concentración sérica de globulina gamma y de mucoproteína en tanto que baja la de albúmina. Sin embargo, estas alteraciones no son diacríticas, dado que también se observaron en cobayos que tenían anomalías no tuberculosas. No hubo ningún paralelismo bien definido entre la elevación de la mucoproteína y

la de la globulina gamma, subiendo principalmente la mucoproteína en la tuberculosis prolongada. Concuerdan bien las concentraciones séricas de globulina gamma, determinadas por métodos químicos y electroforéticos. El valor de la globulina gamma es más alto en los cobayos más viejos que en los más jóvenes.

En las fases latentes de la tuberculosis, producidas por la isonicotidoterapia, la concentración sérica de globulina gamma no sube y la de albúmina no baja. En los animales inoculados con BCG, la seroglobulina gamma se eleva ligeramente y la albúmina desciende ligeramente. La sobreposición de una lesión hepática o de una infección con bacilos de Friedlander no alteró las fracciones de seroproteína en la tuberculosis latente o en los animales vacunados con BCG más allá del efecto de la lesión hepática por sí sola, pero en la tuberculosis se notó effecto acumulativo.

En la tuberculosis experimental, es muy notable la basofilia citoplásmica de los elementos mesenquimatosos en el hígado, de los reticuloendoteliocitos en el bazo y de las células epitelioideas y linfoideas de los granulomas específicos. El paralelismo de la basofilia citoplásmica de estos elementos mesenquimatosos y de la elevación de la globulina gamma no es suficientemente neto para sugerir intensamente la localización en estos elementos de globulina gamma formada excesivamente. Se sugiere que el exceso de globulina gamma se forma a costas de la albúmina.

Resume

Variations de la protéine sérique dans la tuberculose expérimentale

Chez le cobaye tuberculisé, la concentration de la gamma-globuline et des mucoprotéines du sérum s'élève alors que celle de l'albumine s'abaisse. Néanmoins ces modifications ne permettent pas un diagnostic, car on les observe aussi chez les cobayes indemnes de lésions tuberculeuses. Il n'y avait pas de parallélisme net entre l'augmentation de la mucoprotéine et celle de la gamma-globuline, la mucoprotéine s'élevant surtout dans la tuberculose prolongée. Les concentrations de la gamma-globuline sérique déterminées par les méthodes chimiques et électrophorétiques concordent bien. La concentration de la gamma-globuline est plus élevée chez les cobayes-témoins plus âgés que chez l'animal jeune.

Aux stades latents de la tuberculose, produits par l'isoniazidothérapie, le taux sérique de la gamma-globuline n'est pas augmenté et celui de l'albumine n'est pas réduit. Chez l'animal inoculé avec le BCG, la gamma-globuline sérique est légèrement élevée et l'albumine légèrement diminuée. Une lésion hépatique surajoutée ou l'infection par le bacille de Friedlander ne modifiait pas les fractions protéiques du sérum dans la tuberculose latente ou chez l'animal vacciné au BCG au delà de ce qui était imputable à la lésion hépatique seule, mais dans la tuberculose un effet cumulatif était observé.

La basophilie du cytoplasme des éléments mésenchymateux du foie, des cellules réticuloendothéliales de la rate et des cellules épithélioïdes et lymphoïdes des granulomes spécifiques se manifeste de façon frappante. Le parallélisme de la basophilie cytoplasmique de ces éléments mésenchymateux et de l'élévation de la gamma-globuline n'est pas assez net pour corroborer la conception d'une localisation de l'excès de formation de gamma-globuline dans ces cellules. L'article suggère que l'excès de gamma-globuline se produit aux dépens de l'albumine.

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EFFECT OF TWEEN® 80 AND SERUM ON THE INTERACTION OF MYCOBACTERIOPHAGE D-29 WITH CERTAIN MYCOBACTERIAL SPECIES[1,2,3]

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(Received for publication July 16, 1957)

INTRODUCTION

Studies by Groman and Bobb (1) have shown that Tween® 80 prevents plaque formation of diphtheria bacteriophage by preventing adsorption of phage to susceptible bacteria. They also reported that Tween did not interfere with adsorption of Coli phages T_1 and T_2 or with phage *C* on *Bacillus megatherium*.

While attempting to isolate phage-resistant tubercle bacilli in Tween-albumin medium containing mycobacterial phage *D-29* (2), it was noted that the bacteria grew profusely without apparent lysis, but that they were lysed by this phage in media without Tween. Substantially similar results were observed by Redmond (3).

In further experiments in this laboratory it was found that serum prevented the inhibitory action of Tween on phage lysis and that serum concentrations of 10 per cent were optimally effective for this purpose through a relatively wide range of Tween concentrations.

Finally, it was shown that, in addition to prevention of phage propagation, Tween also prevented the development of phage-resistant mycobacteria which regularly appeared in a propagating system in its absence. Serum was found also to counteract this effect of Tween.

In the present report experiments will be described which demonstrate these effects of Tween and serum on the interaction of phage *D-29* and certain species of mycobacteria.

MATERIALS AND METHODS

All tests in which *M. tuberculosis* strain ATCC607[4] was cultivated were performed in heart infusion broth (Difco). Studies with human virulent tubercle bacilli[5] were performed in Dubos albumin medium (Difco) (4). A sterile 1 per cent solution of Tween 80 in heart-

[1] From the George Hunter Laboratory, Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee.

[2] This study was supported, in part, by a grant from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association; Tennessee Tuberculosis Association; and Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York.

[3] Presented before the Medical Session, as part of *Section 1B*, at the annual meeting of the National Tuberculosis Association, Kansas City, Missouri, May 6, 1957.

[4] Mycobacteriophage *D-29* and its propagating strain, *M. tuberculosis* ATCC607, were obtained from Dr. Seymour Froman, Olive View Sanatorium, Olive View, California.

[5] *M. tuberculosis* var. *hominis*, strain H37Rv, was obtained from Dr. William Steenken, Trudeau Laboratory, Saranac Lake, New York; *M. tuberculosis* var. *hominis*, strain No. 115, was isolated in this laboratory in 1956.

infusion broth was kept at 4°C. as a source for preparation of media containing Tween. Sero-negative, complement-inactivated, pooled human serum was used in preparing media containing this material. Serum was heated at 56°C. for thirty minutes, stored at 4°C., and then sterilized by filtration through a Seitz filter before use.

Phage propagation[4] was performed by the semisolid layering method of Gratia (5) and, in a single passage, titers usually attained a concentration of 10^8 or 10^9 plaques per ml. Plaque counts were made by incorporating 0.1 ml. of the dilutions to be tested in semisolid layers and observing for plaque formation after forty-eight hours' incubation. *M. tuberculosis*, strain ATCC607, was used as the indicator strain for all plaque counts. Mycobacterial colony counts were made by dropping dilutions of the organism suspensions to be tested on solid media and incubating until growth was sufficient for enumeration of colonies.

Tests of the effect of Tween and serum on *D-29* phage reactions with *M. tuberculosis* ATCC607 were performed in 5 ml. volumes of liquid media while those with human virulent strains were performed in semisolid layers in a manner similar to phage propagation. Inocula of human tubercle bacilli in these tests were grown in albumin medium without Tween and homogenized in a Teflon grinder before addition to the test medium. Tests with strain ATCC607 were incubated only overnight while those with human strains were incubated for four days. In all cases colony counts and phage plaque counts were made before and after each period of incubation on each of three replicate tests.

Finally, plaque counts were made of preparations of phage *D-29* and mycobacterial strain ATCC607 following incubation for thirty minutes in liquid media containing Tween, serum, or neither of these agents. Further plaque counts were made of the supernatants following centrifugation for ten minutes at 3,000 r.p.m.

Results

Inhibition of phage propagation: When *M. tuberculosis* strain ATCC607 and *D-29* mycobacteriophage were incubated in varying concentrations of Tween 80, it was observed (figure 1, table 1) that in concentrations of 0.0048 per cent and higher no increase in phage titer occurred over the original phage titer of 6.4×10^2 particles per ml. At 0.0024 per cent Tween concentration, slight in-

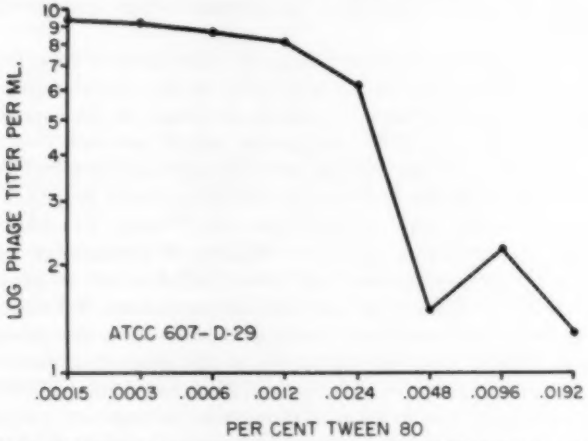

Fig. 1. Effect of Tween 80 on propagation of mycobacteriophage *D-29* with *M. tuberculosis* ATCC607.

TABLE 1

TITER OF D-29 BACTERIOPHAGE AFTER OVERNIGHT INCUBATION WITH ATCC607 IN VARYING CONCENTRATIONS OF TWEEN 80

Per Cent Tween 80	Plaques per Ml.
0.0192	30
0.0096	200
0.0048	50
0.0024	2.1×10^6
0.0012	1.3×10^8
0.0006	6.9×10^8
0.0003	1.5×10^9
0.00015	3.8×10^9

Initial concentration of phage = 640 plaques per ml.
Initial concentration of *M. tuberculosis* ATCC607 = 8.8×10^5 colonies per ml.

hibition of propagation was suggested with 2×10^6 particles per ml. detected after overnight incubation. At Tween concentrations below these values, phage titers reached 10^8 particles per ml. or greater, approximating results in phage propagation systems without Tween.

Effect of serum on the inhibition of phage propagation by Tween: When serum in final concentrations of 10 and 25 per cent was added to tests containing concentrations of Tween previously shown to inhibit phage propagation with strain ATCC607, it was found that phage propagation once again occurred, reaching titers approximating those in systems without Tween or serum (figure 2, table 2). In the experiment shown it may be further observed that there was some deterioration of the phage activity in the presence of Tween, especially at a concentration of 0.01 per cent in the absence of serum. However, phage particles from such systems retained the ability to propagate when transferred to media without Tween.

As Tween is frequently used in media for cultivation of tubercle bacilli, it was of interest to learn whether the growth of inocula of mycobacteria in such media would prevent subsequent propagation of phage. In this experiment, cells of mycobacteria strain ATCC607 were grown in 0.05 per cent Tween, then diluted either 1:10 or 1:100 so that the final Tween concentration in the propagating system was below the threshold of interference with propagation (0.0005 per cent Tween) or just above it (0.005 per cent Tween). In addition, various concentrations of serum were added to aliquots of propagating system containing these cells for comparison with the effect of serum in previous tests.

In figure 3 and table 3 it may be seen that the same effects of Tween and serum previously described occurred once more and that Tween-grown inocula did not differ in these respects from inocula grown in the absence of Tween.

Effect of Tween and serum on the growth of M. tuberculosis ATCC607: In previous studies (figure 3) it was observed that phage propagation was appreciably less in 90 per cent serum than in a serum concentration of 10 or 20 per cent. It

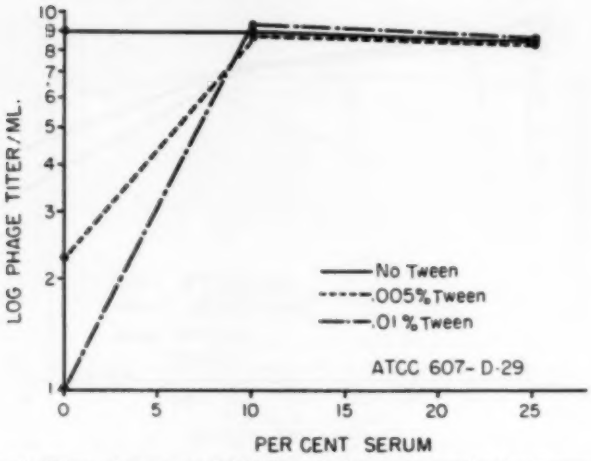

FIG. 2. Effect of serum on inhibition of phage propagation by Tween 80.

TABLE 2

EFFECT OF SERUM ON INHIBITION OF PHAGE PROPAGATION BY TWEEN 80

Per Cent Tween 80	Per Cent Serum Concentration	Plaques per Ml. After Overnight Incubation
0	0	9.9×10^8
0	10	8.6×10^8
0	25	2.5×10^8
0.005	0	2.3×10^2
0.005	10	6.8×10^5
0.005	25	1.6×10^8
0.01	0	10
0.01	10	1.5×10^3
0.01	25	2.9×10^5

Initial concentration of phage = 2.6×10^3 plaques per ml.
Initial concentration of *M. tuberculosis* ATCC607 = 1.3×10^6 colonies per ml.

was suspected that this represented an inhibitory effect of serum on the growth of the propagating strain of Mycobacterium with consequent limitation of phage multiplication. For this reason, enumeration of bacterial colonies (ATCC607) after incubation in media containing various concentrations of serum was done. It was found (figure 4, table 4) that 5 or 10 per cent serum had little effect, and that 50 or 90 per cent serum concentrations significantly interfered with growth. These effects were especially noticeable after ninety-six hours of incubation. In contrast to the effect of serum, Tween in concentrations up to 0.02 per cent

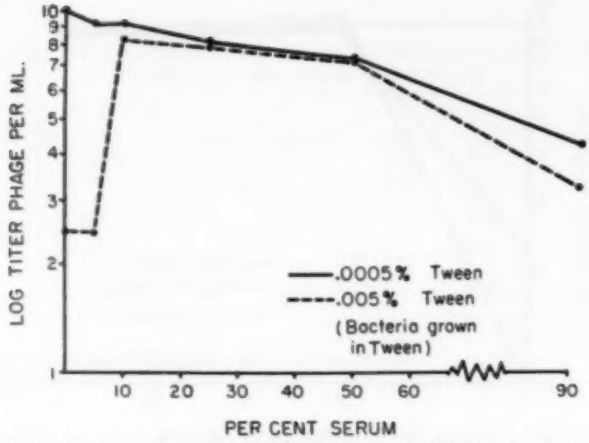

FIG. 3. Failure of growth of mycobacterial inoculum (ATCC607) in 0.05 per cent Tween to alter effects of Tween and serum on propagation of phage *D-29*.

TABLE 3

EFFECT OF GROWTH OF PROPAGATING STRAIN ATCC607 IN 0.05 PER CENT TWEEN MEDIUM ON SUBSEQUENT PHAGE PROPAGATION IN VARYING CONCENTRATIONS OF SERUM

Per Cent Serum	Plaques per Ml.	
	Bacterial Inoculum Diluted 1:10 (0.005 Per Cent Tween Concentration)	Bacterial Inoculum Diluted 1:100 (0.0005 Per Cent Tween Concentration)
0	4.9×10^{9}	1.0×10^{10}
5	4.5×10^{9}	1.1×10^{9}
10	3.2×10^{8}	1.2×10^{9}
25	9.6×10^{7}	1.0×10^{8}
50	1.4×10^{7}	2.7×10^{7}
90	2.1×10^{5}	2.3×10^{6}

Initial concentration of phage = 1.0×10^{3} plaques per ml.

did not interfere with the growth of strain ATCC607 when compared to the growth in media without Tween.

Effect of Tween and serum on the propagation of phage on human tubercle bacilli: A number of experiments were performed in liquid media to judge the effect of Tween and serum on the propagation of phage on human tubercle bacilli. On several occasions results similar to those with *Mycobacterium* 607 were observed. On other occasions no propagation of phage occurred in control media or in those containing Tween and serum. Because of this inconsistency an attempt was made to propagate phage *D-29* on human tubercle bacilli in semisolid

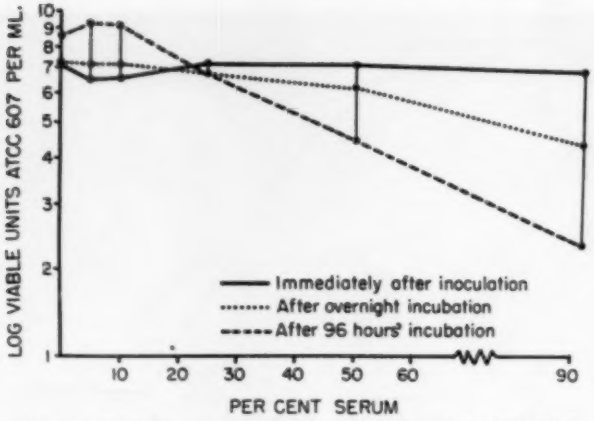

FIG. 4. Effect of serum on growth of *M. tuberculosis* ATCC607.

TABLE 4

EFFECT OF SERUM ON GROWTH OF M. TUBERCULOSIS ATCC607

Per Cent Serum	Bacterial Colonies per Ml.		
	Immediately After Inoculation	After Overnight Incubation	After 96 Hours' Incubation
0	1.0×10^7	2×10^7	6.4×10^8
5	5.0×10^6	2×10^7	1.0×10^9
10	6.0×10^6	2×10^7	1.0×10^9
25	1.6×10^7	8×10^6	8.0×10^5
50	1.2×10^7	2×10^6	5.0×10^4
90	8.0×10^6	$<4 \times 10^4$	3.6×10^2

media. In several experiments with satisfactory controls the inhibitory effect of Tween and its counteraction by serum were observed with human tuberculosis strains H37Rv and No. 115, a fresh isolate from this laboratory. In table 5 and figure 5 one such experiment with H37Rv is described.

Effect of Tween 80 in preventing the appearance of phage-resistant colonies of Mycobacterium 607: It has frequently been observed that propagating systems containing *D-29* phage and mycobacterial strain 607 regularly yielded bacterial colonies resistant to phage lysis. These resistant colonies were round, with regular margins and smooth glistening surfaces (figure 6*B*). In contrast, phage-susceptible colonies had serrated margins and irregular surfaces (figure 6*A*). It was soon found that, in tests with Tween in which no phage propagation occurred, no resistant bacteria could be isolated. In tests containing Tween but also with sufficient serum to permit phage propagation, phage-resistant forms were invariably detected. Colonies of strain 607 resembling those which were

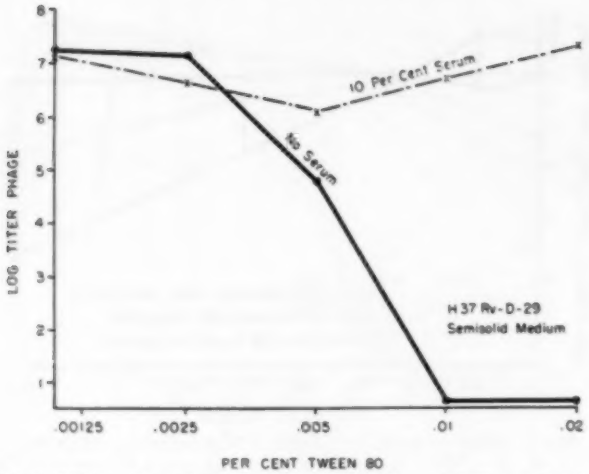

FIG. 5. Effect of Tween 80 and serum on the propagation of *D-29* mycobacteriophage on *M. tuberculosis* var. *hominis* H37Rv in semisolid medium.

TABLE 5

EFFECT OF TWEEN 80 AND SERUM ON PHAGE PROPAGATION WITH M. TUBERCULOSIS H37Rv

Per Cent Tween	Plaques per Ml.	
	No Serum	10 Per Cent Serum
0.00125	2.6×10^7	1.2×10^7
0.00250	1.5×10^7	6.4×10^6
0.00500	8.0×10^5	1.6×10^6
0.01000	<80	5.6×10^4
0.02000	<80	2.5×10^7

Initial concentration of phage = 200 plaques per ml.
Initial concentration of *M. tuberculosis* H37Rv = 1.0×10^6 colonies per ml.

phage resistant were never observed to occur spontaneously in the absence of phage, and those which were phage resistant maintained this characteristic through animal passage and in numerous subcultures in the absence of phage- or Tween. It should be noted that colonies of mycobacterial strain 607 grown on solid media containing Tween superficially resembled phage-resistant colonies, but this appearance promptly changed to the usual colony morphology when transfer was made to media free of Tween.

Effect of Tween and serum on recovery of phage D-29 after centrifugation of phage-mycobacterium ATCC607 preparations: When phage *D-29* and ATCC607 were incubated in heart-infusion broth without serum or Tween for 30 minutes, an average of 62 per cent of the phage particles were removed by centrifugation. When the experiment was repeated in heart-infusion broth with 0.01 per cent

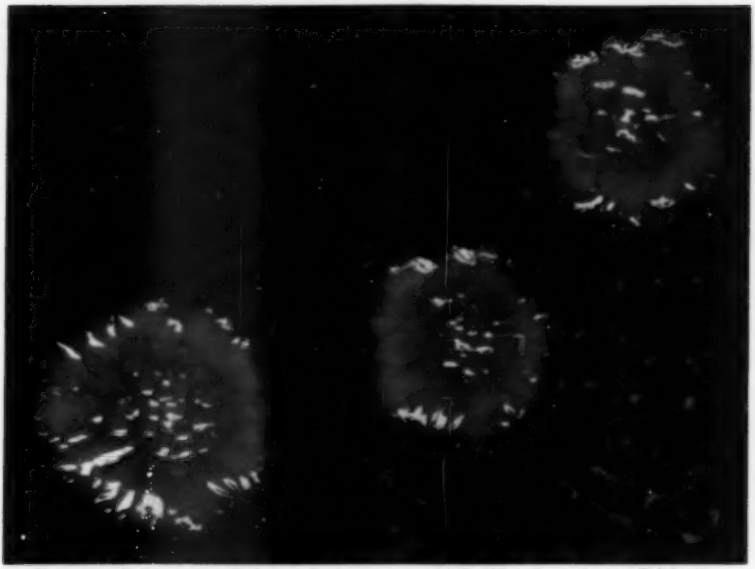

FIG. 6A. Colony morphology of phage-susceptible *M. tuberculosis* ATCC607.

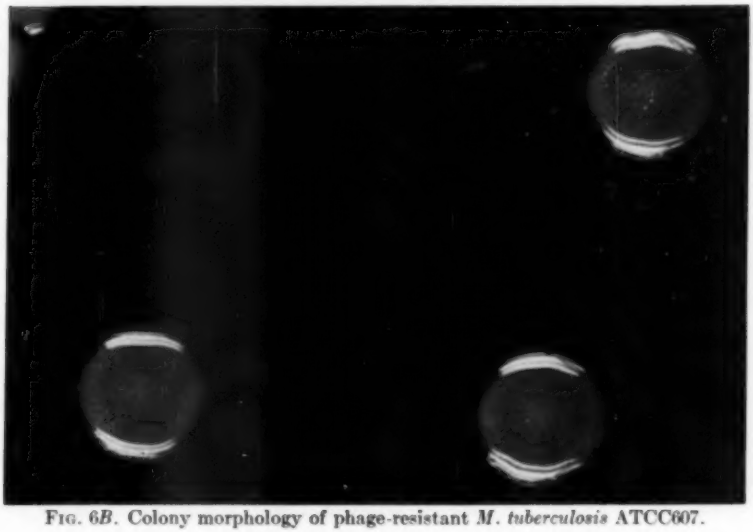

FIG. 6B. Colony morphology of phage-resistant *M. tuberculosis* ATCC607.

Tween, there was no reduction in plaque counts following centrifugation. When 10 per cent serum and 0.01 per cent Tween were present the results obtained were similar to those obtained in media which contained neither Tween nor serum.

TABLE 6

EFFECT OF TWEEN AND SERUM ON RECOVERY OF PHAGE D-29 FROM SUPERNATANTS AFTER INCUBATION WITH MYCOBACTERIUM TUBERCULOSIS ATCC607

	Plaques per Ml.		Per Cent Removed
	Before Centrifugation	After Centrifugation	
No Tween, no serum	4.1×10^5	1.8×10^5	56
	3.8×10^5	1.6×10^5	58
	3.1×10^5	1.2×10^5	61
	6.7×10^3	3.3×10^3	51
	6.7×10^3	3.6×10^3	46
	6.6×10^3	3.2×10^3	52
	410	70	83
	190	20	89
	150	60	60
			—
			Mean 62
0.01 per cent Tween, no serum	3.5×10^5	3.4×10^5	3
	3.3×10^5	3.6×10^5	−9
	2.9×10^5	2.7×10^5	7
	3.8×10^3	4.4×10^3	−16
	3.6×10^3	3.3×10^3	9
	2.7×10^3	2.6×10^3	4
	160	130	19
	80	70	13
	60	50	12
			—
			Mean 5
0.01 per cent Tween, 10 per cent serum	300	60	80
	150	10	94
	110	20	82
			—
			Mean 85

DISCUSSION

The foregoing experiments have revealed that Tween 80 prevents the propagation of mycobacteriophage *D-29* upon two strains of human tubercle bacilli and a saprophytic mycobacterium. It was further demonstrated that serum prevents this effect of Tween. A further manifestation of the interference with phage action by Tween is the failure of phage-resistant forms to appear in media containing this agent, in contrast to their regular occurrence in phage-bacterium systems in non-Tween media.

Of interest in connection with the effect of Tween and serum on phage propagation are the effects of these substances on the susceptibility of tubercle bacilli to antimicrobial drugs. Dubos and Davis (6) have demonstrated that tubercle bacilli grow in a highly dispersed state, often as single cells, in Tween media. In contrast, in liquid media without Tween, tubercle bacilli grow in

clumps containing many cells. Clumped growth of tubercle bacilli in non-Tween medium is highly resistant to the antimicrobial drugs, penicillin and subtilin (7, 8). When tubercle bacilli are in a dispersed state in Tween media, however, they are highly susceptible to these two agents. Serum has been shown to inactivate the effect of Tween in dispersing growth of tubercle bacilli, and it likewise diminishes the effectiveness of the two antimicrobial drugs against these organisms.

It has been assumed that the effect of Tween in increasing the susceptibility of tubercle bacilli to drugs is dependent upon the dispersion of bacterial cells, making them more accessible to drug effect. With the mycobacteriophage phenomenon, however, it appears that Tween in some manner provides a barrier to adsorption of phage. It is suggested that the surface effect of Tween in overcoming a natural tendency toward clumped growth of tubercle bacilli prevents the attachment of phage particles, in contrast to its facilitation of the effect of antimicrobial agents which are in solution in the surrounding medium.

Recently Groman and Parker (9) have reported that diphtheria bacteriophage is adsorbed to the diphtheria bacteria in the presence of oleic acid, but the phage infection is rapidly aborted. An aborted infection is also consistent with the present findings that phage was not removed by centrifugation after thirty minutes' incubation with ATCC607 in Tween media. Further experiments will be needed on this point.

It is also possible that phage itself may be affected by Tween as a basis for the phenomenon described in the present report. While there is no evidence yet available concerning this possibility, it seems less likely than the earlier explanation.

The action of serum to prevent dispersion of growth of tubercle bacilli by Tween may be due to a lipase which is present in both human serum and preparations of bovine serum albumin (10). This enzyme catalyzes the hydrolysis of Tween with the liberation of free oleic acid. Heating albumin preparations at 56°C. for thirty minutes inactivates the lipase (11), but identical treatment of the human serum used in these studies did not prevent the serum from allowing phage propagation to occur in the presence of Tween. While this observation does not eliminate the possibility of hydrolysis of Tween by lipases as an explanation of the effect of serum, it seems to make such an explanation less likely.

An alternative explanation which has been suggested for the interference with Tween by serum is the binding of Tween by serum lipoprotein. The interaction between Tween and serum with regard to dispersion of growth of tubercle bacilli is quantitative, and increasing degrees of dispersion of growth may be produced by increasing the Tween concentration in media containing serum. Thus, dispersed growth may even be obtained in media containing 20 per cent serum when a concentration of 0.4 per cent Tween is present (7).

It is possible that the effect of Tween which results in growth dispersion, and its interference by serum, is also responsible for the effects on phage propagation described in this report. Stoichiometric reactions of this type are more suggestive of binding or chemical combination than they are of enzymatic hydrolysis;

however, no evidence is available on this point and further study of the problem is required.

Knowledge of the action of Tween 80 and serum obtained in this investigation may be used to study phage-mycobacterial relationships in several ways. The following are examples of such uses in this laboratory.

The action of Tween is useful in facilitating the recovery of phage-susceptible mycobacteria from media or tissues containing phage *D-29*. If colony counts are made from organs of mice infected with phage-susceptible mycobacteria and treated with phage, the phage may lyse the bacteria on media which allow phage propagation. From such colony counts it would appear that phage had eradicated the infection. If colony counts are made on media with sufficient Tween to prevent lysis of the bacteria by phage, however, colony counts similar to those in untreated animals are obtained.

Finally phage lysates contain other components of the lysed cells in addition to phage. It is possible that the changes in the bacteria occurring in phage-bacterial mixtures might be due to other materials present in the phage inoculum. The effect of Tween in preventing both propagation of phage *D-29* and changes in morphology and the ability of serum to allow both phage propagation and morphologic changes in media containing Tween all suggest that these changes are due to the action of phage. It may be possible in this manner to utilize the action of Tween and serum to distinguish the action of phage from other materials present in phage lysates in the study of mycobacterial species.

Summary

It has been shown that Tween® 80 prevents the propagation of mycobacteriophage *D-29* on the otherwise phage-susceptible mycobacteria, *M. tuberculosis*, ATCC607, H37Rv, and a freshly isolated human virulent strain. This effect apparently resulted from interference by Tween with adsorption of phage to host bacterium. Pooled human serum in a concentration of 10 per cent or more prevented this action of Tween.

Tween 80 prevented the appearance of phage-resistant strains of *M. tuberculosis* ATCC607, a phenomenon which regularly occurred when *D-29* and a phage-susceptible culture of ATCC607 were incubated without this agent. Serum also counteracted this effect of Tween.

Sumario

El Efecto del Tween 80 y del Suero sobre la Interacción del Microbacteriófago D-29 con Ciertas Especies Microbacterianas

Se ha demostrado que el Tween® 80 impide la propagación del micobacteriófago D-29 en las micobacterias que de otro modo serían fago-susceptibles, *M. tuberculosis*, ATCC 607, H37Rv, y en una cepa humana virulenta, recién aislada. Este efecto provino al parecer de la obstaculización por el Tween de la adsorción del fago en la bacteria hospedadora. El suero humano combinado, a una concentración de 10 por ciento o más, impidió esta acción del Tween.

El Tween 80 impidió la aparición de cepas fago-resistentes del *M.* tuberculosis, ATCC 607, fenómeno este que ocurrió con regularidad cuando se incubaban sin dicho agente el D 29 y

un cultivo fago-susceptible de ATCC 607. El suero también contrarrestó este efecto del Tween.

Resume

Effets du Tween 80 et du Sérum sur l'interréaction du Mycobactériophage D-29 et de certaines espèces mycobactériennes

Il a été démontré que le Tween® 80 prévient la propagation du mycobactériophage *D-29* sur les mycobactéries, *M-tuberculosis*, ATCC 607, H37Rv et sur une souche humaine virulente récemment isolée, habituellement phago-sensibles. Cet effet résultait apparemment d'une interférence du Tween dans l'activité d'adsorption du phage vis-à-vis de l'hôte bactérie. Le sérum "pooled" à la concentration de 10% ou plus prévenait cette action du Tween.

Le Tween 80 prévenait l'apparition de souches phago-résistantes du *M. tuberculosis*, ATCC 607, phénomène qui se manifestait régulièrement lorsque de *D-29* et une culture phago-sensible de ATCC 607 était incubée sans cet agent. Le sérum s'opposait également à cet effet du Tween.

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STUDIES ON THE CATALASE ENZYME OF MYCOBACTERIA[1]

I. Correlation Between Catalase Activity and Virulence of Mycobacteria Under Certain Experimental Conditions

OTTÓ SCHWEIGER, ELVIRA ROMÁN, AND I. SOÓS

(Received for publication July 25, 1956)

INTRODUCTION

It has long been known that catalase is produced by mycobacteria, as well as by other aerobic bacteria. Recently it was shown (1, 2) that isoniazid-resistant strains of tubercle bacilli no longer produce catalase. It was also demonstrated (1, 2) that catalase-negative strains are less pathogenic for guinea pigs. These data suggest the possibility of a certain correlation between catalase activity and the virulence of mycobacteria. The purpose of the present study is to determine the rate of catalase activity of various mycobacteria, including virulent, avirulent, and saprophytic strains, under certain experimental conditions.

MATERIAL AND METHODS

The following strains were used:

Virulent Strains

H37Rv: The cultures used for catalase activity tests were fourteen to sixteen days old.

Bd2: Virulent bovine strain. The cultures used were twenty-six to thirty-two days old.

B: Virulent bovine strain. This strain was isolated in 1938 by Kertay from the liver of a cow.

Ravenel: The cultures used were twenty-six to thirty-two days old.

Avirulent Strains

BCG: Ten-day-old cultures of the original Calmette strain were used.

Vole (A 178): This strain was obtained by Kertay from A. Q. Wells in 1949. The cultures used were twenty-eight days old.

H37Ra: Avirulent human strain. The cultures used were twenty-eight days old.

E: Fully avirulent human strain. Isolated originally in 1890 by Ehrlich in Paris, this strain was obtained in 1937 by Kertay from Plum. The cultures used were twenty-eight days old.

K and *Y:* Fully avirulent bovine strains, both obtained by Kertay from Plum in 1937. The cultures of these two strains used for catalase activity tests were twenty-one days old.

Saprophytic Strains

M. smegmatis, *M. butyricum*, *M. Rabinowitsch*, *M. Frieburgensis*, and *M. phlei*. These strains were provided by the Microbiological Institute of Lausanne. All were used as four-day-old cultures for the catalase activity tests.

Culture medium: Sauton culture medium was used in all of the experiments described.

Catalase activity tests: The various mycobacteria were filtered, washed thrice with saline, and diluted in saline so that the resulting suspension contained 50 mg., wet weight, of the

[1] From the Koranyi Tuberculosis State Sanatorium, Budapest 124, Hungary.

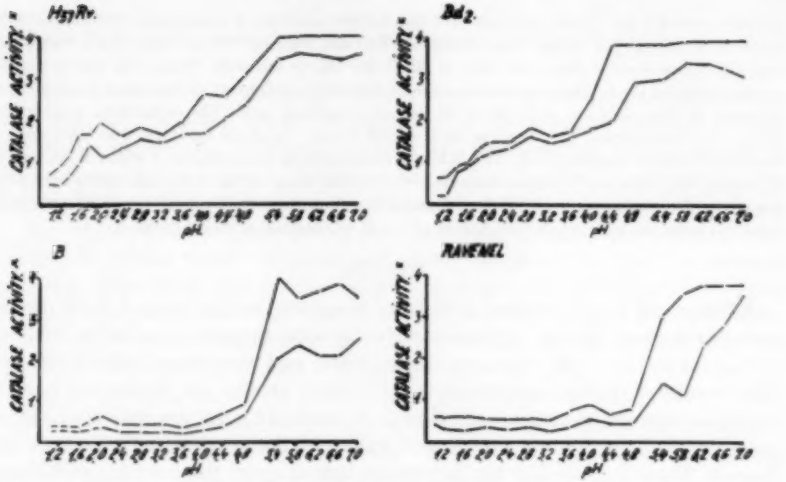

FIG. 1. The rate of catalase activity of virulent strains of mycobacteria at various pH levels (extreme values).

* Catalase activity is expressed in terms of potassium permanganate consumption.

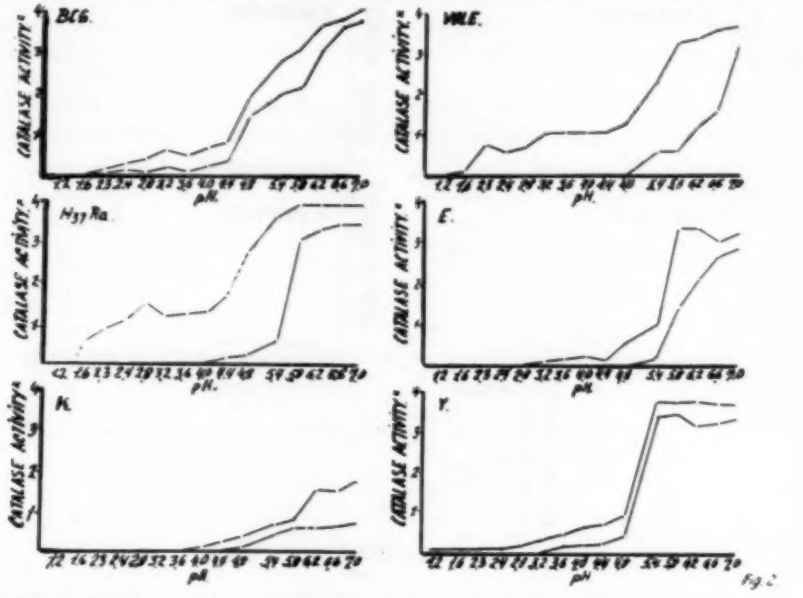

FIG. 2. The rate of catalase activity of avirulent strains of mycobacteria at various pH levels (extreme values).

* Catalase activity is expressed in terms of potassium permanganate consumption.

bacteria per 0.5 ml. After addition of 2 ml. buffer solution, a suspension with a bacterial content of 50 mg. per 2.5 ml. was obtained. To this, 1 ml. of 697 per cent H_2O_2 was added and the tubes were shaken and kept at 20°C. for thirty minutes. Then, the process of decomposition of H_2O_2 into water and oxygen (due to the presence of bacterial catalase) was stopped by the addition of 1 ml. of 20 per cent sulfuric acid. The remaining quantity of H_2O_2 was determined by titration with $n/10KMnO_4$. Catalase activity was expressed in terms of $KMnO_4$ consumption. The $KMnO_4$ consumption was measured when H_2O_2 was titrated in the absence of mycobacteria (A), furthermore when titration occurred in the presence of mycobacteria (B). The difference between these two values (A and B) is equivalent to catalase activity in milliliters. ($A - B$ = catalase activity in ml.)

Results

Effect of pH on the catalase activity of mycobacteria: In figure 1 is shown the rate of catalase activity of virulent mycobacteria suspended in buffer solutions of various levels of pH. Sörensen citrate buffer and phosphate buffer were used. The results of similar experiments for avirulent strains are illustrated in figure 2 and for saprophytic strains in figure 3. At each pH point are indicated the two extremes, i.e., the highest and lowest values of the catalase activity tests performed. These consisted of five determinations at every pH level for each strain. This number was raised at the critical pH points, where catalase activity tended to become zero, to as much as ten to fifteen tests. In figure 4 the results illustrated

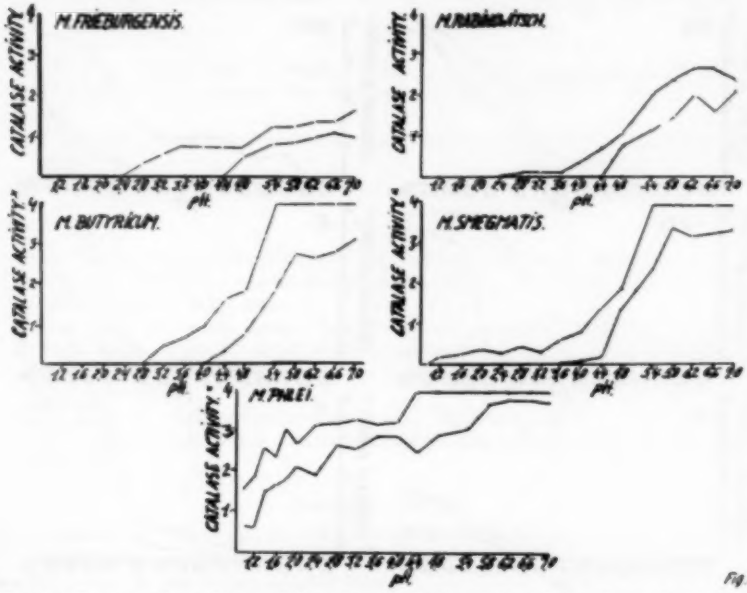

FIG. 3. The rate of catalase activity of saprophytic strains of mycobacteria at various pH levels (extreme values).

* Catalase activity is expressed in terms of potassium permanganate consumption.

in figures 1, 2, and 3 are summarized and the average values for these are calculated.

As may be seen, all strains manifested a nearly identical rate of catalase activity from pH 7 to pH 5.4, with the exception of the avirulent strain *K* and the saprophytic strains *M. Frieburgensis* and *M. Rabinowitsch*, which showed a decreased amount of activity. Beginning at pH 5.4, the curves representing the catalase activity of avirulent strains tended to drop more rapidly than those for the virulent strains. Thus, before pH 1 was reached, all of the avirulent and saprophytic strains except *M. phlei* had ceased completely to produce catalase.

On the other hand, the virulent strains and also the saprophytic *M. phlei* showed a less pronounced reduction in catalase activity at the lower pH levels. As a result, virulent strains such as H37Rv, virulent bovine *B*, *Bd2*, and the saprophytic strain *M. phlei* were still catalase active at pH 1.

Effect of temperature on the catalase activity of Mycobacteria: The effect of temperature on catalase activity was studied by suspending the Mycobacteria (50 mg. per 2.5 ml.) in phosphate buffer of pH 7 and placing them in water baths of various degrees of temperature for ten minutes. Catalase activity was determined as described above. In figure 5 the extreme values of catalase activity tests are shown which were performed on virulent bacteria kept previously at different temperature levels. The results of similar studies for avirulent bacteria are illustrated in figure 6, and for saprophytic strains in figure 7. A summary of the results presented in figures 5, 6, and 7 with calculation of the average values is shown in figure 8.

In figure 8 it may be seen that the avirulent strains were no longer catalase active at 60°C. The virulent strains became catalase negative from 64°C. to

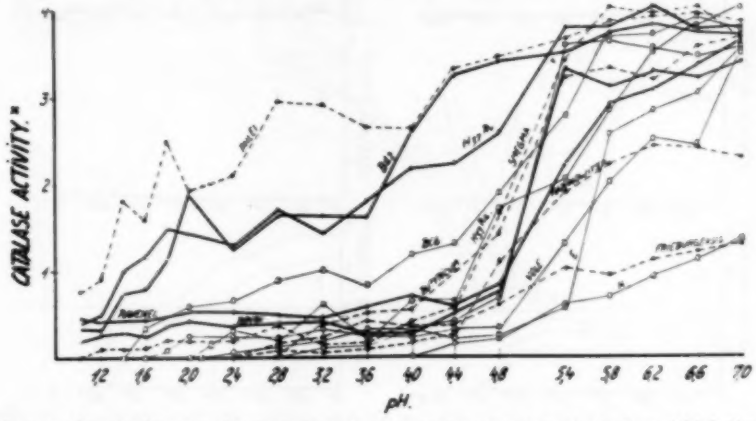

FIG. 4. Graph showing the average rate of catalase activity of virulent (thick line), avirulent (thin line), and saprophytic strains (dotted line) of mycobacteria at various pH levels.

* Catalase activity is expressed in terms of potassium permanganate consumption.

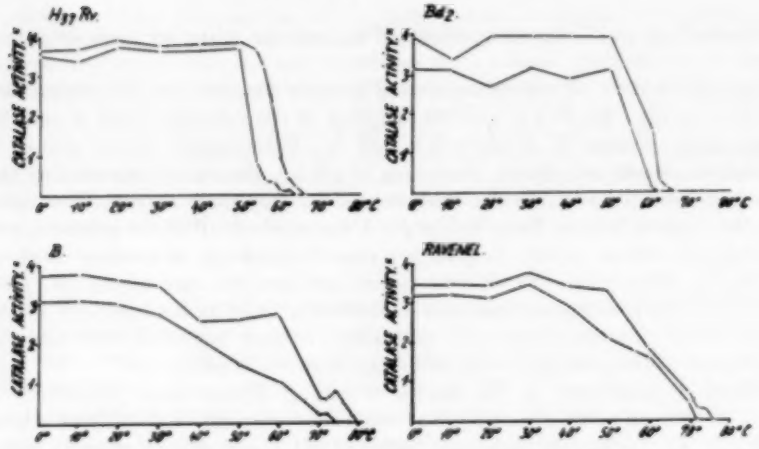

FIG. 5. The rate of catalase activity of virulent strains of mycobacteria at various temperature levels (extreme levels).

* Catalase activity is expressed in terms of potassium permanganate consumption.

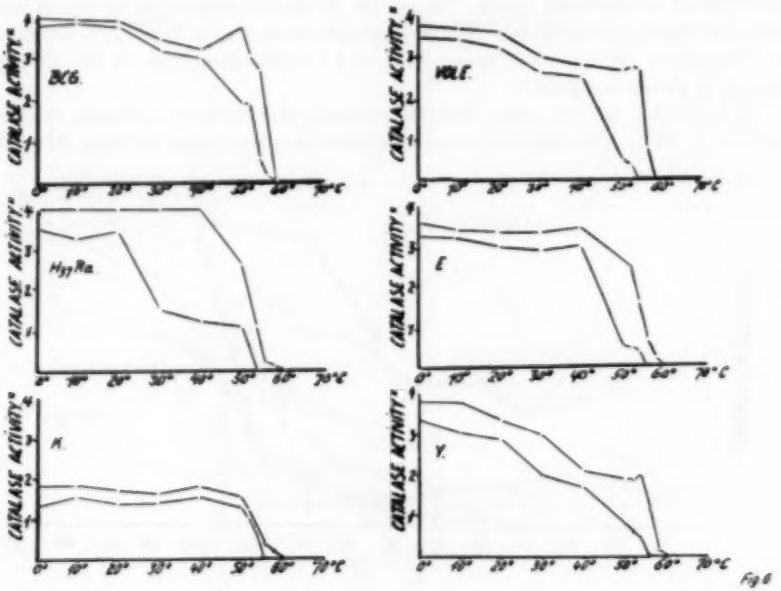

FIG. 6. The rate of catalase activity of avirulent strains of mycobacteria at various temperature levels (extreme values).

* Catalase activity is expressed in terms of potassium permanganate consumption.

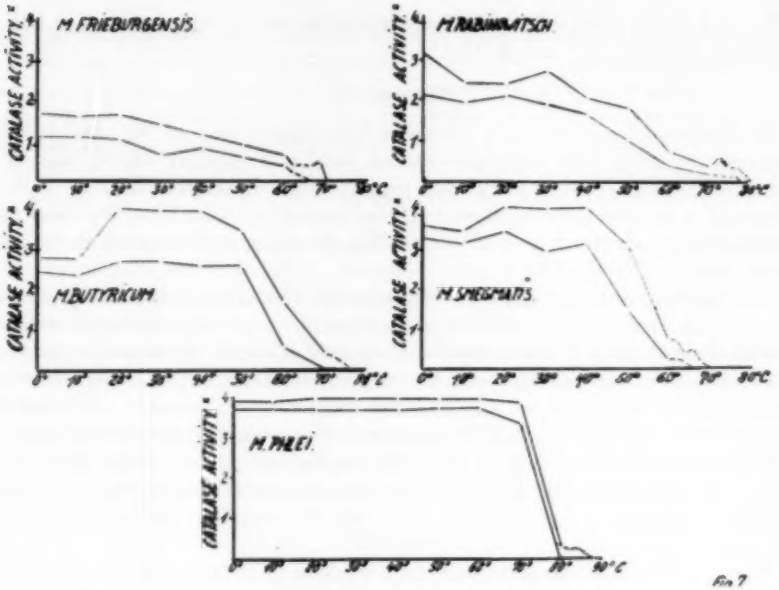

FIG. 7. The rate of catalase activity of saprophytic strains of mycobacteria at various temperature levels (extreme levels).

* Catalase activity is expressed in terms of potassium permanganate consumption.

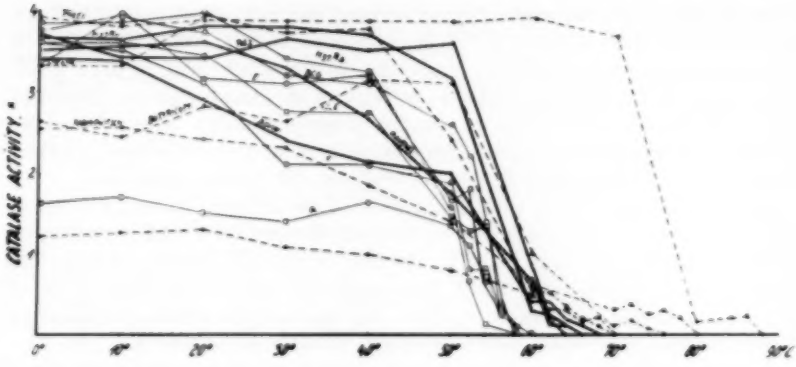

FIG. 8. Graph showing the average rate of catalase activity of virulent (thick line), avirulent (thin line), and saprophytic strains (dotted line) of mycobacteria at various temperature levels.

* Catalase activity is expressed in terms of potassium permanganate consumption.

68°C. and the catalase activity of the saprophytic strains was abolished at temperatures between 70° and 88°C.

Discussion

In the recent literature much discussion has centered around two phenomena observed to occur with isoniazid-resistant strains of tubercle bacilli, namely, decrease in pathogenicity for guinea pigs and loss of catalase activity (1–5). Although a parallel between these two phenomena has been found by most of the investigators, the degree of relationship between them appears to be far from clear.

As a first step of a systematic investigation into the catalase-virulence problem the present writers have conducted experiments with mycobacterial strains previously unexposed to the antibacterial effect of isoniazid. In these experiments the behavior of the catalase enzyme of virulent, avirulent, and saprophytic strains (all of them without any previous contact with isoniazid) was studied under certain experimental conditions, such as changes in the pH or temperature. The results have shown that at pH 7 the catalase activity of strains BCG, *M. phlei*, *M. smegmatis*, and H37Rv is identical in degree despite the fact that H37Rv is pathogenic and BCG, *M. phlei*, and *M. smegmatis* are nonpathogenic for guinea pigs.

These data would appear to indicate a uniformity in the catalase activity of virulent, avirulent, and saprophytic strains and thus to negate the existence of catalase-virulence correlation. With the modification of pH, however, a difference was revealed between virulent and avirulent mycobacteria. This is well documented by the data in figure 4. From pH 7.0 to pH 5.4 the various strains tested showed an approximately equal amount of catalase activity, except for the strains of bovine avirulent *K*, *M. Frieburgensis*, and *M. Rabinowitsch*, which are somewhat less active catalase producers. On the other hand, at pH 1 a difference may be found in the catalase activity of the various strains. While at such a pH level a perfect lack of catalase-producing ability is manifested by avirulent and saprophytic strains except *M. phlei* (a saprophytic strain), virulent strains such as H37Rv, *Bd2*, *B*, and Ravenel are still catalase producers.

Another difference in the catalase activity of the different strains was found after their exposure to various degrees of heat. The results of such experiments (figure 8) clearly show that at 60°C. the rate of catalase activity of avirulent strains is zero. On the other hand, virulent strains are still catalase active at 60°C. and continue to be so up to 68°C. For saprophytic strains, inactivation of the bacterial catalase by heat takes place between 70° and 88°C.

On the basis of these observations, it is evident that the catalase enzyme of virulent, avirulent, and saprophytic strains reacts differently to various test conditions. Such a difference in reaction is probably due to the behavior of the protein component of the enzyme (apoferment) which is known to be highly specific for the various microbial species (6). It would appear that the protein component of the enzyme is inactivated in the different strains to a varied degree when exposed to various levels of pH or heat.

These data suggest the possibility of using the difference in the catalase activity of the various mycobacteria at pH 1 and at 60°C. as a basis for determining their virulence similarly to the methods known as cytochemical reaction tests. However, in view of the fact that the strains used in this study were laboratory strains, the necessity for further studies must be emphasized.

Summary

Virulent strains of mycobacteria (H37Rv, *Bd2*, *B*, Ravenel) and *M. phlei* are still catalase active at pH 1. Avirulent mycobacteria (H37Ra, BCG, vole, *K*, *Y*, *E*) and the saprophytic strains (*M. Frieburgensis*, *M. smegmatis*, *M. butyricum*, and *M. Rabinowitsch*) manifest no catalase activity at pH 1.

Inactivation of the bacterial catalase by heat occurs between 64° and 68°C. in the virulent strains. Avirulent mycobacteria are no longer catalase active at 58° to 60°C.

Saprophytic strains cease to produce catalase from 70° to 88°C.

It is suggested that the rate of catalase activity of the various mycobacteria at pH 1 and at 60°C. might be a useful measure to test their virulence.

Sumario

Estudios de la Encima Catalasa de las Micobacterias

Cepas virulentas de micobacterias, tales como H37Rv, *Bd2*, B y Ravenel, y el saprofito *M. phlei* son todavía catalaso-activos a un pH 1. Micobacterias avirulentas, tales como H37Ra, BCG, *Microtus*, K, Y y, E, y los saprofitos *M. frieburgensis*, *M. smegmatis*, *M. butyricum* y *M. rabinowitsch* no manifiestan actividad catalásica a un pH 1.

La inactivación al calor de la catalasa bacteriana tiene lugar entre 64° y 68°C. Las micobacterias avirulentas ya muestran actividad catalásica a 58° a 60°C. Las cepas saprofitas dejan de mostrarse activas en catalasa a 70° a 88°C.

Apúntase que el tenor de la actividad catalásica de las varias micobacterias a un pH 1 y a 60°C. podría constituir una medida util para comprobar la virulencia de las mismas.

Resume

Recherches sur l'enzyme catalase des Mycobactéries

Les souches virulentes de mycobactéries telles que H37Rv, *Bd2*, B, Ravenel, et le M. phlei saprophyte manifestent encore au pH 1 une activité de la catalase. Les mycobactéries avirulentes, telles que H37Ra, BCG, vole, K, Y, E et le *M. Frieburgensis*, saprophyte, *M. smegmatis*, *M. butyricum* et *M. Rabinowitsch* ne manifestent au pH 1 aucune activité de la catalase.

L'inactivation de la catalase bactérienne par la chaleur apparaît entre 64°C. et 68°C. L'activité de la catalase des mycobactéries avirulentes disparaît à 58–60°C. L'activité de la catalase des souches saprophytes cesse à 70–88°C.

L'article suggère que le taux d'activité de la catalase à pH 1 et à 60°C. chez les diverses mycobactéries pourrait être une mesure utile pour tester la virulence bactérienne.

Acknowledgment

The writers are grateful to Dr. N. Kertay, Microbiological Institute, Budapest, for providing avirulent strains, Vole (A 178), *K*, and *Y*, and virulent strain *B*.

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REPORT ON INTERNATIONAL UNION AGAINST TUBERCULOSIS[1]

JAMES E. PERKINS[2]

The time was the fall of 1948. The place: the little auditorium of the headquarters of the National Tuberculosis Association of France—a gift of the Rockefeller Foundation at the close of World War I—across the street from the famous Luxembourg Gardens of Paris. The occasion: the end of the first session of the Council of the Union.

The place was in an uproar. The delegation from the National Tuberculosis Association of the United States was clamoring for a vote on a resolution it had suddenly presented to the Council calling for a broadening of the program of the Union, clarification of relationships with the new World Health Organization (which had come into being less than six months earlier), and providing for the employment of a full-time executive director of the Union. The president was in a quandary. He could not permit an immediate decision on such a revolutionary proposal shattering at one stroke the traditions of many years. He precipitously adjourned the meeting.

The interval between that session and the concluding session of the Council later in the week was filled in by the scientific sessions and by elaborate receptions, luncheons and dinners, which provided opportunities for hurried off-the-record conferences, explanations, and some "horse trading." This resulted in the working out of a slightly modified resolution, which was presented at the concluding session of the Council—and adopted!

RESOLUTION PASSED UNANIMOUSLY BY THE COUNCIL OF THE INTERNATIONAL UNION AGAINST TUBERCULOSIS, PARIS, SEPTEMBER 29, 1948

WHEREAS

(*a*) The time has come for the International Union Against Tuberculosis to take a more active part than hitherto in antituberculosis measures throughout the world, and to be the one recognized international voluntary agency in tuberculosis, and whereas,

(*b*) There is an urgent need for an increase in the number of national tuberculosis associations and for the encouragement of further effort by existing national tuberculosis associations, and whereas,

(*c*) There should be established a close, effective cooperation between the World Health Organization and the International Union, and whereas,

(*d*) The coordination of governmental and voluntary effort in the antituberculosis campaign is an essential and urgent function of the Union, and whereas,

(*e*) Adequate financial resources should be obtained in order for the Union to carry out a comprehensive international campaign against tuberculosis,

IT IS RESOLVED:

1. To hereby establish the new position of full-time Executive Director in the Union.
2. To designate the Executive Committee as a Committee with full power to select

[1] Presented at the luncheon at the time of the meeting of the National Tuberculosis Association in Kansas City, Missouri, on May 9, 1957.

[2] Managing Director, National Tuberculosis Association.

and appoint as soon as possible a qualified physician to this full-time position of Executive Director to carry out the principles embodied in the preamble to this resolution, with this officer to be paid an annual salary commensurate with the heavy responsibilities involved, and with full and complete executive power under the general supervision of the Executive Committee and Council.

3. That this same committee investigate and attempt to solve the existing financial problem of the Union, including exploration of financial assistance from sources in addition to the member national associations.
4. And that the constitution of the International Union be further considered in the light of these proposals by the committee already set up for this purpose and that a revised constitution be submitted to the Council at the meeting to be held in 1949, prior to submission to the general meeting in Copenhagen in 1950.

Thus, the way was paved for the International Union Against Tuberculosis to become a truly significant force in the control of tuberculosis throughout the world and a more effective medium for the exchange of scientific information concerning all aspects of the etiology and therapy of tuberculosis.

The principal compromise reached during the interval negotiations was to accept the continuation of the position of Secretary-General, with the understanding that this would continue to be a part-time honorary position, but that in addition a full-time executive director would be employed to handle the administrative details of the anticipated expanded program.

The blunt upstart from the Midwest, who presented the resolution for the National Tuberculosis Association delegation, deserved little credit, other than brashness stemming from ignorance of the slowness of grinding of international wheels and failure to realize the desirability (well known in diplomatic circles) of never saying precisely what one really means but saying it, nevertheless, beautifully and at great length. His only prior experience in international councils had been at the first World Health Assembly earlier that same summer; but this at least had given him an opportunity to get to know Dr. J. B. McDougall, who had headed the tuberculosis work of the WHO Interim Commission and who was continuing as chief of the Tuberculosis Section of the now permanently established WHO.

The original resolution concerning expansion of the Union's program actually had been drafted by Dr. McDougall as a suggestion of what he believed was needed for clarification of relationships between WHO and the Union in the tuberculosis field, and also as an indication of his feeling the need for a strong voluntary international tuberculosis agency to build up citizen support in various countries for adequate tuberculosis control programs which would be stimulated simultaneously by WHO through the governments of those same countries. The original draft was modified by the National Tuberculosis Association delegates with help from the delegates from Great Britain, and some other countries, before presentation.

The successful negotiations in the interval between the Council sessions, resulting in final acceptance of the resolution, were accomplished largely through the activities of the other four representatives that year on the Council from the National Tuberculosis Association—Kendall Emerson, who with his suave charm,

impeccable English, and less impeccable but helpful French, calmed the fears of the elder statesmen; Esmond R. Long, who assured his many intimate friends from other countries that this would not jeopardize but rather would elevate the scientific stature of the Union; James R. Rueling, who put to good use his skill and experience in negotiating delicate issues in the circles of organized medicine; and Herman E. Hilleboe, whose facile and convincing statement before the Council smoothed the way for the resolution's passage; and last, but not least by any means, the expert advice of Frederick D. Hopkins inserted unobstrusively, per usual, at precisely the right moment.

It wasn't that the Union was markedly deficient. It had been an extraordinarily superior and effective organization in the past. It had been established in 1920 after World War I as the result of a conference the previous year of a distinguished group of men, including Dr. Charles James Hatfield of the United States (whose memory we are honoring in the principal presentation this noon by Professor Arvid Wallgren), Professor Leon Bernard of France, and Sir Robert Philip of Great Britain. Professor Leon Bernard was appointed Secretary-General, and biennial conferences of importance were held in various countries until World War II forced the Union into hibernation for the duration.

This Union was itself really a successor to an earlier one, dating back to the turn of the century and which had its headquarters in Berlin—but at the end of World War I, of course, anything smacking of Germany was anathema. Attempts by the Germans to revive the original organization were boycotted by representatives of the allied countries, and a new organization—the current Union—was established by them instead.

The earlier German-dominated organization, known as the International Anti-Tuberculosis Association, was responsible to a large extent for the creation of the National Tuberculosis Association in this country at the particular time it was formed. The international association had scheduled an international tuberculosis conference to be held in Paris in 1905 and it was necessary for delegates worthy of representation of the United States to be designated. As a matter of fact, as Dr. Robert G. Paterson, winner of the Will Ross Medal last evening, described so well in his articles in the first and second issues of the National Tuberculosis Association Historical Series, there were two so-called "national" organizations concerning themselves with tuberculosis at the time, but neither embraced the top leaders in medicine and public health of that era, and relationships between the two were strained, to say the least. Furthermore, it seemed important as a stimulus to the promotion of dynamic programs of control of tuberculosis in the United States to hold an outstanding international conference on the subject in this country, and the existing organizations were considered incapable of doing so. Lawrence F. Flick of Philadelphia felt strongly that the solution was establishment of a new National Association for the Study and Prevention of Tuberculosis rather than building up one of the two already in existence, as suggested by some of his colleagues. Flick's stubborn tenacity again prevailed, and thus the National Tuberculosis Association came into existence in 1904. The delegates representing the association at the conference in Paris in 1905 in-

cluded such prominent men as Flick, William Osler of Baltimore, Hermann M. Biggs, S. Adolphus Knopf of New York City, Vincent Y. Bowditch of Boston, John H. Lowman of Cleveland, Arnold E. Klebs of Chicago, and Francis Pottenger of California. They issued an invitation to hold the next conference in the United States, and the United States was host at a very successful international conference in Washington, D. C. in 1908, headed by President Theodore Roosevelt himself, and managed by Lawrence Flick.

Thus, it is clear that from the very beginning the National Tuberculosis Association has been very much interested in the international as well as domestic aspects of the control of tuberculosis, and our bylaws clearly state that one of the objectives of the Association is the promotion of "international relations in connection with the study and control of tuberculosis."

Following World War II the Union was a little slow to revive and adjust to new world-wide changes and needs—partly due to the terrific disruption of affairs because of the war, and due in part to the ill health of the Secretary-General, Professor Fernand Bezancon (Leon Bernard's successor), which resulted in Professor Bezancon's death in the summer of 1948. It was thus at the Council meeting that same fall of 1948 that the time seemed ripe for a revival and modernization of the Union to meet the new international challenges. These resulted from the upsurge of tuberculosis throughout much of the world because of the war, the availability of effective drugs which had just been developed, the new and compelling interest in international cooperation resulting from the development of the atomic age, and the creation of the United Nations and its specialized agencies. The resolution calling for expansion of the Union to come abreast of these developments opened the door to such rehabilitation of the Union.

But it merely opened the door, it didn't automatically force entry—and steps toward it faltered. To tempt compliance with the intent expressed in the resolution, the National Tuberculosis Association the next year offered to match the combined grants of other members of the Union to a total of $10,000, if there were employed a suitable full-time executive director. The staff at the time consisted of a part-time secretary whose minutes frequently bore little resemblance to what had transpired at a given meeting. The total budget of the Union was about $2,000 per year. The meetings of the Union prior to 1948 were conducted without the benefit of an interpreter; you knew French fluently or else! As a matter of fact, it didn't make too much difference if you couldn't understand or speak French because there were no votes put to the Council, and there were no committees in existence except the Executive Committee, which made all of the decisions and passed them on to the Council for its information.

Progress was sufficiently slow so that, at the time of the first post-war conference in Copenhagen in 1950, the NTA delegation and others who shared in the desire to improve the influence of the Union felt rather frustrated. The program for that conference followed the same formalized pattern which had been the tradition for the thirty years of the Union's existence. Although several outstanding papers were presented, the conference primarily provided the oppor-

tunity for the clinician or surgeon to talk about his cases and his success in treatment, without the troublesome inconvenience of presenting evidence meeting minimum requirements of scientific validity to substantiate his conclusions. No opportunity was provided for the exchange of ideas and information on administrative aspects of tuberculosis control programs. No serious attempt as yet had been made to employ an executive director, and the NTA's $10,000 was still resting securely in the bank in the United States while unkind comments were made from time to time by others in the Union regarding the failure of the NTA of the richest country in the world to support the program of the Union, except for the piddling amounts coming in from individual memberships.

Knowing our unhappiness, the Department of State of the United States had requested the delegation from the United States to the Copenhagen Conference to do nothing rash at that session because of the then delicate international situation—apparently a permanent state of affairs. I must confess I was a little skeptical that the tensile strength of the spinal column of the international camel had been calibrated so precisely that the added straw of disruption of the International Union would be just enough to precipitate collapse.

During the preliminary business sessions in Copenhagen in 1950, in which the intents of the resolution passed in 1948 were to be incorporated into the constitution of the Union, a conservative block of the elder statesmen of the Union were successful in toning down the provisions of the resolution.

It was at this low point in the morale of the representatives of the United States that a suggestion expressed in the typical drawl of a citizen of Kansas City, the late Dr. Herbert L. Mantz, came to the rescue. I can see "Pete" now, inserting another cigarette in his long holder, cocking his head on one side and saying slowly, "You know!—what you need in this outfit is an international NCTS!" Of course! Why hadn't we thought of it before? An organization of the chief executives of the member national tuberculosis associations of the Union could not only be a most potent stimulus to improving and enlarging the activities of the Union, but would also provide the medium for exchange of ideas and information on the administrative aspects of tuberculosis control programs so neglected in the past; just as the National Council of Tuberculosis Secretaries, now the National Council of Tuberculosis Workers, had been so helpful in the United States with reference to the National Tuberculosis Association. On the spot, the NTA called an informal meeting of the executives present from other countries who, with but one or two exceptions, endorsed the idea with enthusiasm. The first Conference of Executive Directors was held the following year, 1951, in Paris under the chairmanship of Dr. Harley Williams, Secretary-General of the National Association for the Prevention of Tuberculosis of Great Britain. It was a great success and the Union had taken one more forward step.

The new Secretary-General, Professor Etienne Bernard, had now had an adequate opportunity to become oriented and realized the time available to him outside his extensive private practice, his teaching at the University of Paris, and the social and professional obligations of a member of a distinguished family of France, was insufficient to carry on the tasks indicated by the new and ex-

tended responsibilities of the Union. He began an intensive search for a suitable full-time executive director. Snags were encountered, but finally in October, 1953, a director was appointed, Dr. William Gellner, a German who was now a British citizen, with extensive international experience in the tuberculosis field with United Nations Relief and Rehabilitation Administration and the International Refugee Organization, and able to speak several languages.

It should also be said at this point that Professor Bernard had progressively earned the admiration and respect of the members of the Union. He had revealed himself to be a man of culture and charm, a man of complete integrity and great diplomatic skill, sound in his medical concepts, and able and willing to adjust his points of view as his experience widened.

This appointment of an Executive Director was only three and one-half years ago. In the meantime the international conferences have progressively improved, with the conference in New Delhi, India, four months ago, a new highwater mark in stature and maturity. The very fact that the meeting was held in India, instead of Europe, is one indication of the extent to which the Union has grown up and is now truly an international body. The conference was attended by some 900 delegates from 60 countries from all parts of the world, including Russia and Communist China, but with a refreshing absence of political insinuations; the delegates had only one enemy, the legions of General *Mycobacterium tuberculosis*, and were indeed a Union working together in planning their strategy of attack against this common enemy. We had, for the first time, adequate facilities for simultaneous interpretation—whatever the meeting room and wherever you sat, you merely had to put on your head set and dial your language releasing the top minds of the world in the tuberculosis field from the chains of the barrier of language, and allowing free interchange of ideas. This permitted the first panel discussions and symposia conducted at a Union conference, which were definitely successful.

Other aspects of the program of the Union also have been improved:

Technical committees have been established, which are beginning to come forth with helpful statements on technical problems of universal import.

Successful international conferences on technical subjects—such as BCG and photofluorography—have been held, sponsored by the Union.

Regional committees have been established in South America, the Middle East, and, just four months ago in New Delhi, for the Southeast Asia region. These are "Operations Bootstrap," in which countries of an area of more or less uniform cultural backgrounds and similar problems assist each other in the establishment of national tuberculosis associations and in improving the programs of those already in existence.

The constitution has been modified to make the Union a more democratic organization.

The publications have been improved.

Better quarters have been obtained to house the central office in Paris.

The budget has increased to almost $40,000 per year, but with the NTA's annual contribution not increasing, since additional countries have joined the

Union and their added contributions have permitted expansion, with the NTA now paying a relatively smaller proportion of the total—a sound trend.

This doesn't mean the Union is in need of no further improvement. Some members of the NTA delegations, who have had an opportunity to attend only one or two sessions, have wondered if any progress really is being made at all. Their impatience is understandable as they have not had the opportunity to observe personally the marked advances evident from observation of a number of years. They forget that the ponderous mechanisms of international clocks revolve at a slower pace than the tiny gears of our individual wrist watches.

But there are needs—and great needs. The international conferences still leave much to be desired. The technical committees have not realized their potentialities. More regional committees are indicated, and with improvement of existing ones. Still better liaison between WHO and the Union is needed. The staff and budget of the Union are still pitifully small.

Progress *is* occurring, however—very heartening progress. Further progress will occur from patient plodding and prodding. Withdrawal of support at this point because of mere impatience with the speed of improvement will accomplish nothing except retrogression of the international program.

And finally we must all remember—just as no home is safe from tuberculosis until every home is safe, no country is safe from tuberculosis until every country has its tuberculosis problem under control.

Case Reports

BRONCHOLITHIASIS[1,2]

Produced by Histoplasmosis

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(Received for publication June 15, 1957)

INTRODUCTION

Expectoration of calcified particles has been known and reported for many years. The first report was by Petersen in 1885 (1). Although not a rarity in clinical practice, the event is not common. The presence of calcium in the hilar lymph nodes is one of the most frequently seen abnormalities in chest films, but symptoms resulting from this calcium are relatively rare.

Review of Literature

In 1914 Paunz (1) reported 14 cases of expectorated broncholiths. He quoted Ghon's figures of 30 impending, recent, or scarred nodal bronchial perforations among 169 autopsies of tuberculous children. He also added that up to 1914 only 8 cases of expectorated broncholiths had been reported.

Terplan (2), in a discussion of occlusion of bronchi in children by tuberculous nodes and foci, described a case of a 2-year-old child from whose bronchus fibrotic remnants of an eroding node were expectorated and tubercle bacilli were identified. This material, however, was not calcified.

Schwarz (3, 4) described numerous cases of perforation of tuberculous lymph nodes in children, frequently with subsequent bronchogenic dissemination. He also observed the healing of a massive perforation in a 9-year-old child.

Fox and Clerf (5) reported 10 cases of broncholithiasis in 1945, and more recently Groves and Effler (6) reviewed their experience in Cleveland with this entity, including 27 cases. In neither of these series was the etiology of the broncholiths demonstrated directly. In the latter report the frequency of pneumonia and bronchiectasis secondary to the obstruction of the involved bronchi was emphasized. Their finding that hemoptysis and cough were the two most common symptoms agrees with the clinical experience of others.

The pathogenesis of the clinical situation reported here has been clarified by the above work and has been summarized by Amberson (7). Due to the development of a primary infection in the lung, the hilar nodes become involved. If the process leads to necrosis and caseation, calcification may result. The involved nodes may become very large and actually compress the soft bronchi of children, resulting in collapse of the distal lung segment. This extrinsic compression seen in children does not occur so often in adults because the adult bronchial tree is more rigid, due to the presence of more cartilage, and the nodes do not get as large in adults as they may in children. In adults, however, because the adenitis is almost always accompanied by periadenitis which tends to fix the bronchi as scar tissue forms, the calcified node that is applied firmly to the bronchus may erode through. (This may occur in children in addition to the compression mentioned above.) The erosion inevitably causes obstructive symptoms related to the mass of the node which is present in the bronchial lumen. In addition, bleeding and irritation due to the trauma of the bronchial wall may

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[2] This work was supported, in part, by grant E-576 of the National Institutes of Health, U. S. Public Health Service.

occur. The breaking off of calcified material leads to expectoration of broncholiths. After the offending node has been extruded the ulceration in the bronchial wall heals. The possibility then remains that cicatricial stenosis will eventually occur.

It had long been accepted that calcification in the lung or hilar nodes invariably meant tuberculosis. Since the 1940's this impression has been shown to be in error, especially in that part of the country known as the Mississippi Valley. Beginning with the experience of the Armed Forces (8) and evolving to the work of Christie (9), the association of pulmonary parenchymal and nodal calcifications with positive histoplasmin skin tests and negative tuberculin skin tests in the endemic area has shown that histoplasmosis is more apt to be responsible for pulmonary calcification than is tuberculosis. Christie's work was enlarged and corroborated by Palmer (10). This impression was proved by the anatomic work of Straub and Schwarz (11) who demonstrated that the skin test positivity was consistent with their finding of *Histoplasma capsulatum* in 67 per cent of routine autopsies done in the endemic area for histoplasmosis.

The anatomic demonstration of the etiologic agent of pulmonary calcification was rare indeed until recently and to the writers' knowledge there has been no report of this being done in relation to expectorated broncholiths. The advent of special staining techniques which demonstrate fungi in tissues has aided greatly the diagnosis of histoplasmosis histologically. The two stains that have been most helpful in the opinion of the present writers are the Gridley and the Grocott procedures (12, 13). By the use of these methods in the cases presented, the exact etiology of the broncholiths was determined.

Case Reports

Case 1. A 53-year-old white male executive was admitted to the hospital on August 26, 1956, with the chief complaint of hemoptysis of several hours' duration. Five days before admission he had complained of right anterior chest pain radiating to the right shoulder. A chest roentgenogram obtained two days before the hemoptysis revealed a slight increase in density over the right upper lobe and several 3 to 5 mm. calcifications in the right hilar and upper right parahilar regions (figure 1). These calcifications had been present and static for at least two years preceding the acute episode. His past history included two separate episodes of myocardial infarction in 1945 and 1948, and a documented duodenal ulcer. There was no significant pulmonary history.

Physical examination revealed a rectal temperature of 100.8°F., inconstant right basilar rales, sinus tachycardia of 100 per minute, and a grade I systolic apical murmur. Laboratory data included a leukocyte count of 12,100 with 81 per cent polymorphonuclears and normal bleeding and clotting times. Several sputum and gastric cultures for *M. tuberculosis* were negative. Chest roentgenograms suggested a faint radiopacity in the right hilum. The right hilar and parahilar calcifications were unchanged. Anteroposterior tomograms of the right lung showed no evidence of cavitation. The skin reactions to first-strength PPD and histoplasmin were positive while the blastomycin and coccidioidin skin reactions were negative. Bronchoscopy was done and revealed a slight degree of hyperemia of the orifice of the apical segmental branch of the right upper lobe bronchus. The bronchogram was normal.

Fever and hemoptysis subsided with no specific treatment, and the patient felt well enough to be discharged from the hospital on September 18, 1956. He remained without symptoms and returned to work. On October 2, 1956, without warning, he coughed up an irregularly shaped broncholith 5 mm. in diameter. He also produced a small amount of white mucoid sputum streaked with blood. This disappeared in a few days. Chest roentgenograms, including tomograms, were taken after the episode (figure 2) and showed the absence of one of the calcific densities present on the previous films. This was a consistent finding, and the general appearance of the missing calcification on the previous films was the same as the described appearance of the broncholith that the patient had expectorated. In addition there was clearing of the right apical density.

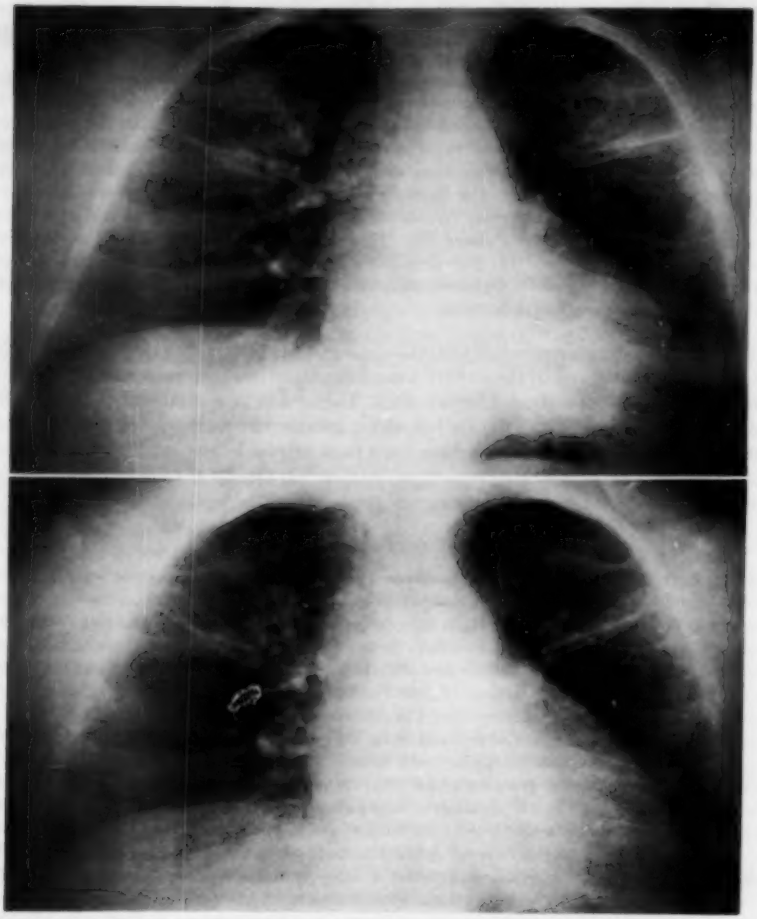

FIG. 1, *Case 1* (Upper). Lordotic chest roentgenogram, showing multiple calcifications and infiltrate, right upper lobe, prior to expectoration of broncholith.

FIG. 2, *Case 1* (Lower). Lordotic chest roentgenogram, obtained after expectoration of broncholith. Note the absence of one calcification and diminution of infiltrate in right upper lobe.

Microscopic study of the stone following decalcification revealed amorphous material which on Gridley stain showed yeast cells morphologically identical with *Histoplasma capsulatum* (figure 3). Stains for acid-fast bacilli were negative.

Case 2. A 50-year-old white female had severe chronic cough for seven to eight years before admission. During this time there were frequent bouts of pneumonia with colds. In 1951 she experienced her first hemoptysis and was hospitalized for it. Bronchoscopy at that time revealed a calcified node eroding through the right main stem bronchus. No further procedures were done at that time. The symptoms continued unchanged until about two

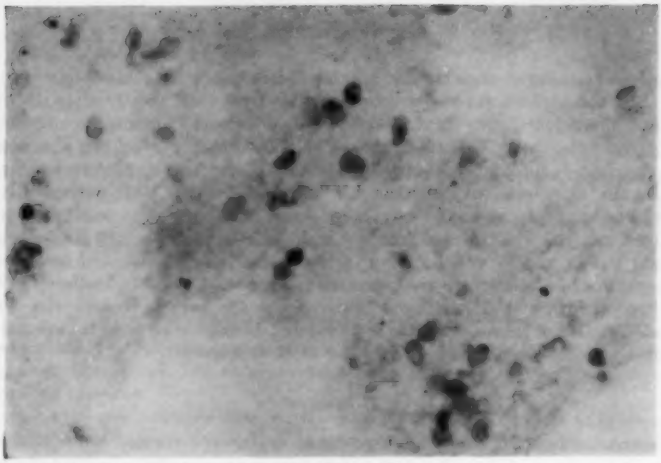

FIG. 3, *Case 1*. Microscopic section of decalcified broncholith expectorated by patient, showing organisms anatomically identical with *Histoplasma capsulatum*. (Grocott stain, 1,000 ×)

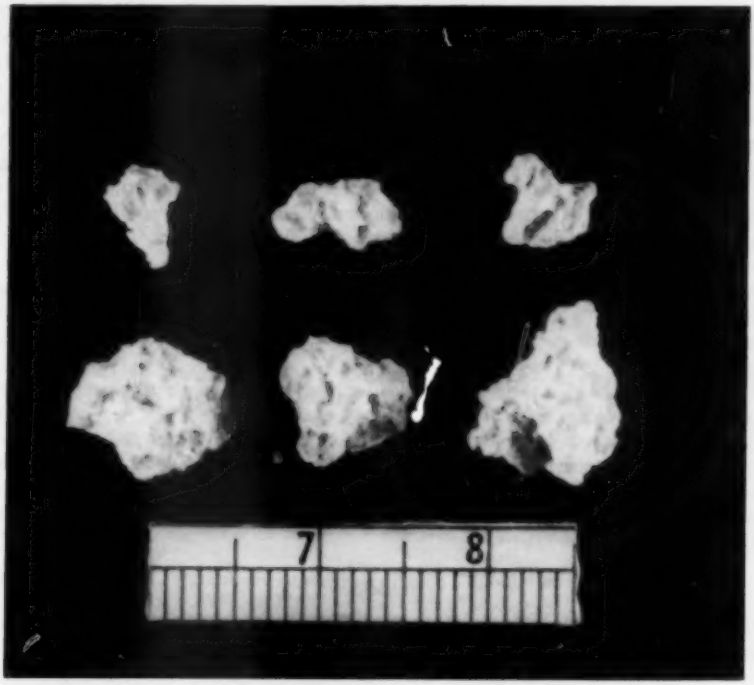

FIG. 4, *Case 2*. Six of the broncholiths expectorated by the patient between hospitalizations. One of these showed organisms on stain morphologically identical with *Histoplasma capsulatum*.

months prior to admission when she had another hemoptysis and coughed up some calcified material. She had expectorated several broncholiths from 1951, shortly after the bronchoscopy, until admission (figure 4). No new symptoms occurred until her admission to the hospital early in March, 1957. At that time the only abnormality noted was a large calcified node in the right hilum and in the right upper lung field on the postero-anterior chest roentgenogram. Skin tests with histoplasmin and first-strength PPD were positive; skin tests with blastomycin and coccidioidin were negative. The patient was bronchoscoped, and calcified material was removed from the right main stem bronchus. Subsequently she had a right middle and lower lobectomy for bronchiectasis, the calcified node being left *in situ* because of its relation to the pulmonary artery wall. Decalcification and Gridley stain of the calcified material removed bronchoscopically revealed yeast cells typical of *H. capsulatum*. One of the broncholiths expectorated during the interval between hospitalizations also revealed organisms characteristic of *H. capsulatum*.

Comment

The demonstration of *Histoplasma capsulatum* in the expectorated broncholiths proves what has been implied by all of the data concerning pulmonary calcifications in the endemic area of histoplasmosis. This has significance in several aspects of intrathoracic disease.

Erosion of a bronchus or severe stenosis of the bronchus by calcified nodes are serious anatomic insults. Because of the frequency of recurrent pneumonia, bronchiectasis or lung abscess distal to an area of stenosis, the hemoptysis, persistent cough, and bronchorrhea secondary to a bronchial erosion, surgical therapy is often indicated in these situations. Resection of lung distal to the bronchial complication appears to be the procedure of choice if bronchoplasty is not feasible. Since the nodes may be incompletely calcified, caseous material may be present which adds the possibility of intrabronchial dissemination of caseous nodal material. The gravity of this situation depends entirely on the etiology of the calcification and caseation. If viable tubercle bacilli are liberated, serious active infection may result. This circumstance indicates specific chemotherapy. If the cause is histoplasmosis, the likelihood of spread of active infection is unknown. The experience of the present writers with this type of case is limited.

Since histoplasmosis can cause eroding broncholiths, a calcified histoplasmic node may cause pressure phenomena at other crucial areas in the thorax. The present writers have actually observed compression of vessels and have also observed an esophageal traction diverticulum.

In areas where histoplasmosis is not endemic these same observations have been made. In these instances tuberculosis is the most likely cause (14).

The most important principle demonstrated is that calcification in the thoracic cage must generally be considered a sequela of inflammation with several possible causes. Since the implications of the various causes are quite different, especially when complications are encountered, every effort should be made to arrive at a correct etiologic diagnosis.

Summary

Two cases of expectorated broncholiths, which were proved to be caused by infection with *Histoplasma capsulatum*, are presented, with the therapeutic and prognostic implications of this finding. Calcification in the chest is most frequently a sequela of granulomatous inflammation caused by one of several agents.

Sumario

Broncolitiasis Producida por Histoplasmosis

Preséntanse 2 casos de expectoración de broncolitos, que resultaron ser producidos por infección con *Histoplasma capsulatum*, señalándose las connotaciones terapéuticas y pronósticas de este hallazgo.

La calcificación torácica es con la mayor frecuencia una secuela de inflamación granulomatosa provocada por varios agentes.

Resume

Broncholithiase produite par l'histoplasmose

Présentation de deux cas avec expectoration de calculs bronchiques dus à l'infection par l'*Histoplasma capsulatum*. Les applications thérapeutiques et pronostiques de cette découverte sont exposées.

La présence de calcifications dans le poumon représente le plus souvent la séquelle d'un processus inflammatoire granulomateux causé par un ou plusieurs agents infectieux.

Acknowledgments

The writers wish to express appreciation to Dr. O. Herman Dreskin for permission to use *Case 1*, and to Dr. Leonard Gottesman for permission to use *Case 2*.

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POSSIBLE SUPERINFECTION WITH ISONIAZID-RESISTANT TUBERCLE BACILLI[1]

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(Received for publication June 24, 1957)

INTRODUCTION

The communicability of tuberculosis has been demonstrated by extensive studies among hospital and sanatorium personnel and among family contacts of known cases. In many institutions, however, it has been assumed that the tuberculous patient has acquired sufficient immunity to protect him from exogenous infection by his fellow patients. Evidence for or against this assumption is meager. In animals it can be demonstrated (1–4) that the original infection imparts a certain degree of immunity against superinfection, but that the immunity is only relative and can be overcome by a sufficiently large inoculum.

It is difficult to prove that superinfection occurs in a patient who already has tuberculosis, since, with few exceptions, strains of human tubercle bacilli cannot be distinguished from each other with certainty. Drug-resistant strains, however, offer convenient markers with which to identify superinfection. Several instances have been reported (5, 6) in which patients with previously existing but untreated pulmonary tuberculosis have developed new disease or exacerbations of old disease associated with the isolation of drug-resistant tubercle bacilli. However, to the writers' knowledge no case of superinfection has been reported in a patient who was receiving chemotherapy for active disease. Reported herein is the case of a patient who apparently became superinfected with catalase-negative isoniazid-resistant organisms while he was receiving streptomycin and para-aminosalicylic acid (PAS).

CASE REPORT

H. E., a 49-year-old white male, was admitted to this hospital on June 24, 1955. He had been well until March, 1955, when he noted dull aching pain in the right side of the chest. He continued working, but two weeks later developed a moderate cough which became productive of a slight amount of sputum during the month prior to admission to the hospital. During the six months prior to admission the patient had lost 55 pounds in weight which he attributed to "overwork" as a dental technician. Two weeks prior to admission he attended the County Clinic for a chest roentgenogram, which showed pulmonary disease. The skin reaction to mid-strength PPD was positive, and the patient was hospitalized accordingly.

He remembered having chest pains in his teens, and once was told he had "pleurisy." A rectal fistula was diagnosed one year ago, and a right inguinal hernia was noted two years ago. The patient was unmarried and lived with his mother. There was no history of tuberculosis, diabetes, or hypertension in the family. For the past twenty-five years the patient had worked as a dental technician; prior to that he had worked as an electrical maintenance man and in a dental supply house.

Physical examination on admission revealed an emaciated, chronically ill, middle-aged male. There were no abnormal signs in the chest. Roentgenograms showed disease throughout both lung fields. At the extreme bases the shadows were discrete, but in the upper parts of both lungs they were conglomerate and dense. There was a 5 cm. cavity in the right apex and there were multiple cavities in the left upper third. Three successive sputum smears

[1] From the Department of Medicine, Cleveland City Hospital; and Western Reserve University School of Medicine, Cleveland 9, Ohio.

and cultures revealed the presence of acid-fast bacilli; one culture was tested and found to be completely susceptible to streptomycin, isoniazid, and PAS. On June 28, 1955, treatment was started with streptomycin (1 gm. daily given intramuscularly) and PAS (12 gm. by mouth daily). Two weeks later the streptomycin dosage was decreased to 1 gm. twice weekly.

The patient became asymptomatic and gained weight steadily. During the year following admission the infiltration showed progressive clearing and the cavities became smaller; but there remained a 3.5 cm. cavity in the right apex and a 2.5 cm. cavity in the left apex. A marked shrinkage of the right upper lobe was noted. Subsequently the roentgenograms showed no appreciable change. A summary of the sputum bacteriology is presented in table 1.

It may be seen that the sputum remained positive for acid-fast bacilli. The organisms, susceptible to all three drugs just prior to the start of treatment, showed slight resistance to isoniazid in February, 1956, and good growth in 30 γ per ml. five weeks later, although it was verified that the patient had not received isoniazid. This was established by careful questioning of the nursing staff and the patient himself and by examination of the nurses' order sheets. The sputum culture of January, 1957, again yielded bacilli which were resistant to isoniazid, and in addition, the culture was catalase negative and nonvirulent for subcutaneously inoculated guinea pigs. Because of the poor clinical response the drug regimen was changed to isoniazid and PAS in April, 1956, but no change in the clinical status was noted after this maneuver. It was not known at that time that the sputum contained isoniazid-resistant organisms.

The patient was in a large solarium during the first two months of his hospitalization, but thereafter he was located in a 16- by 17-foot three-bed room. Both of his roommates

TABLE 1

BACTERIOLOGIC STUDIES OF SPUTUM

Drug Treatment	Date	Examination for Tubercle Bacilli		Drug Susceptibility*		
		Microscopy	Culture	SM	PAS	INH
Streptomycin and	6-27-55	Positive	Positive	S	S	S
	8-10-55 and 9-2-55	Negative	Not done			
PAS	10-7-55 to 2-3-56 (× 5)	Positive	Not done			
	2-26-56	Positive	Positive	SR	SR	SR
	3-2-56	Negative	Not done			
	4-4-56	Negative	Positive	MR	S	HR
Isoniazid and	5-4-56 to 11-12-56 (× 6)	Positive	Not done			
PAS	10-15-56	Positive	Negative			
	12-18-56	Positive	Positive			
	1-2-57	Positive	Positive†	S	SR	MR
	1-6-57 and 2-9-57	Positive	Positive			

* S—susceptible; SR—slightly resistant; MR—moderately reistant; HR—highly resistant (resistant to 100 γ of streptomycin per ml., 25 γ of PAS, or 5 to 10 γ of isoniazid).

† Organisms were catalase deficient and nonpathogenic for guinea pigs.

had far advanced, open tuberculosis, and had been treated for prolonged periods with streptomycin, PAS, and isoniazid. One of them had sputum which showed acid-fast bacilli on microscopy but which was negative on culture, while the other had persistently positive sputum cultures and the tubercle bacilli were highly resistant to isoniazid, moderately resistant to streptomycin, and slightly resistant to PAS. The latter patient was noted to be careless in the handling of his cough and the disposal of his sputum.

Isoniazid-resistant bacilli were first recovered from the patient in the case reported herein five months after his move to the three-bed room; at that time these bacilli apparently made up only a small proportion of the population, since a relatively scanty growth occurred on the drug-containing medium. Later the population consisted almost entirely of isoniazid-resistant, catalase-negative organisms. It is interesting to note that resistance to streptomycin was apparently lost nine months after the drug had been discontinued.

Comment

The demonstration of isoniazid-resistant, catalase-deficient tubercle bacilli in a patient who did not receive isoniazid, and whose culture was susceptible to isoniazid at the start of streptomycin-PAS therapy, is fairly good evidence of superinfection. It is admitted that the case would be stronger if the initial susceptibility had been confirmed by another pretreatment culture, but there was nothing unusual about the case at that time, and only one culture was tested. It would also be more convincing if it were known that the April, 1956, culture was catalase negative, but the catalase activity was not determined. The only other explanations for the observed facts might be that the patient took isoniazid surreptitiously or that the infection with drug-resistant organisms occurred at an earlier date and only became manifest later on. Neither of these explanations, however, seems very likely.

If the premise of superinfection is accepted, it is worthy of note that the new infection was produced by bacilli which are supposed to have a reduced infectivity for humans (7). In addition, the incident emphasizes once again the communicability of tuberculosis even among patients with active disease.

Summary

A case is presented of apparent superinfection in a patient with active pulmonary tuberculosis who was being treated with streptomycin and PAS. The bacilli of superinfection were marked by their catalase deficiency and isoniazid resistance and could have had their source in infection from a careless roommate.

Sumario

Sobreinfección con Bacilos Tuberculosos Isoniacidorresistentes

Preséntase un caso de aparente sobreinfección en un enfermo que tenia tuberculosis pulmonar activa bajo tratamiento con estreptomicina y PAS. Los bacilos de la sobreinfección se caracterizaban por su deficiencia en catalasa y resistencia a la isoniacida, pudiendo proceder de un compañero de cuarto, descuidado.

Resume

Surinfection à bacilles tuberculeux isoniazido-résistants

Présentation d'un cas de surinfection manifeste chez un malade atteint de tuberculose pulmonaire en activité en cours de traitement par la streptomycine et le PAS. Ces bacilles de surinfection étaient caractérisés par leur déficience en catalase et leur résistance à

l'isoniazide. Leur origine pouvant être attribuée à une contamination due à la négligence d'un malade de la salle.

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Notes

WHAT DOES TUBERCULOSIS COST?

An Estimate for Fiscal 1956

At least $725 million was spent for tuberculosis in the United States and its territories in 1956. This is an increase of more than $100 million over the $621 million estimate made by the Public Health Service for the fiscal year 1952.[1] The major differences between the 1952 estimate and the 1956 estimate are: an increase of $85 million in hospitalization expenditures, an increase of $22 million in compensation expenditures, and a decrease of $36 million in construction expenditures. Moreover, in 1956 for the first time the survey included reports from psychiatric hospitals. This information alone added nearly $25 million to the total estimate.

These estimates are based on: (*1*) information collected from federal agencies, state and local governments, and nongovernmental organizations; (*2*) data available as a result of other operations of the Public Health Service; and (*3*) published data about hospital facilities. These data were assembled, analyzed, and the estimates were made by staff members of the Tuberculosis Program of the Public Health Service, Department of Health, Education, and Welfare.

Agencies reporting from the federal government are the departments of Agriculture; Defense; Health, Education, and Welfare; Justice; Labor; State; and the Veterans Administration. Included under the Department of Health, Education, and Welfare are the Office of Vocational Rehabilitation, the Public Health Service, and the Social Security Administration.

The state and local government category contains expenditures of state and local health and welfare departments, and public hospitals.

Nongovernmental organizations include the National Tuberculosis Association and its affiliates, private foundations, colleges and universities, pharmaceutical research laboratories, and private and nonprofit hospitals.

In evaluating these estimates, it is important to realize that considerable sums paid by individuals not in hospitals to private physicians for medical care are not included. In addition, costs of administration in many cases have not been included because of the difficulty of segregating them. Consequently, the estimate of $725 million for tuberculosis control is very conservative (table 1).

Hospitalization and rehabilitation: Of the total expenditures, more than half, $443 million, was spent on hospitalization and rehabilitation. Since many hospitals consider tuberculosis rehabilitation an integral part of patient care and do not report it as a separate cost item, the two areas are considered as a single unit. In estimating expenditures, the costs of outpatient care provided by hospitals and inpatient care are both included.

In 1952 an estimated $332 million was spent on hospitalization and rehabilitation. The increase in the cost of hospitalization and rehabilitation is chiefly responsible for the increase in the total cost of tuberculosis care.

In making a comparison with the 1956 figures, however, a number of factors have to be taken into consideration. The most important is the changed nature of tuberculosis con-

[1] An estimate: Tuberculosis expenditures in fiscal 1952 for the United States and Territories, Pub. Health Rep., 1954, *69*, 797.

TABLE 1

Estimates of Expenditures for Tuberculosis
United States and Territories, Fiscal Years 1952 and 1956

	1956	1952
	In Thousands of Dollars	
Hospitalization and rehabilitation	443,162	332,672
Federal government	124,252	108,657
(Public Health Service)	(13,805)	(2,295)
State and local governments	292,925	195,284
Non-governmental	25,985	28,731
Compensation	211,382	189,254
Federal government	198,569	175,174
(Public Health Service)	(—)	(—)
State and local governments	12,813	13,666
Non-governmental	—	414
Construction	2,055	37,725
Federal government	1,618	9,034
(Public Health Service)	(27)	(5,044)
State and local governments	427	25,008
Non-governmental	10	3,683
Research	3,961	4,396
Federal government	2,430	1,281
(Public Health Service)	(1,576)	(1,151)
State and local governments	—	178
Non-governmental	1,530	2,938
Control	58,174	53,855
Federal government	10,627	12,866
(Public Health Service)	(6,444)	(8,413)
State and local governments	29,503	25,069
Non-governmental	18,045	15,921
Other	6,318	3,121
Federal government	—	—
(Public Health Service)	(—)	(—)
State and local governments	1,544	—
Non-governmental	4,774	3,121
Total	725,052	621,023
Federal government	337,496	307,012
(Public Health Service)	(21,852)	(16,904)
State and local governments	337,212	259,204
Non-governmental	50,343	54,807

Note: The changes in the estimates between 1952 and 1956 are largely due to differences in actual expenditures. However, in several categories the changes are due in substantial part to differences in the kind and quality of data available. For a discussion of the meaning of changes in specific categories, see the appropriate part of the text.

trol and care. New drugs and improved surgical techniques have been so effective that the average number of tuberculosis beds occupied has been reduced. At the same time the cost of operating tuberculosis hospitals has gone up.

The cost of hospitalization depends on these two interrelated variables—the patient-day cost and the number of patients hospitalized each day. Although the number of hospitalized patients declined from 1952 to 1956, this was more than offset by the resulting increase in unit cost, bringing on, in 1956, an increase in the total cost of hospitalization. The increase in expenditures resulting from these factors amounted to about $86 million from 1952 to 1956. In addition, the 1956 estimate includes nearly $25 million for the care of tuberculous patients in psychiatric hospitals, not estimated in 1952.

Of the $443 million spent on hospitalization and rehabilitation in 1956, approximately $124 million were federal funds, the greatest part of which went for hospital care of tuberculous patients in federal institutions. Payments to nonfederal hospitals and sanatoriums for the care of federal beneficiaries are also included. The Public Health Service's share of the federal expenditures was nearly $14 million.

In the fiscal year 1955–1956 state and local governments spent nearly $239 million for hospitalization and rehabilitation. Independent organizations spent about $26 million. Expenditures of both groups were computed from the number of beds occupied by tuberculous patients in nonfederal hospitals and the estimated cost per patient-day. The 1956 census made by the Public Health Service of hospitals with tuberculosis beds was the source of information for the number of occupied beds. The cost per patient-day was estimated from data contained in *Hospitals*, the journal of the American Hospital Association.[2]

The estimate of funds spent by nongovernmental organizations is definitely conservative since expenditures by some foundations for rehabilitation have not been listed because of lack of available data. Estimating the amount spent for hospitalization and rehabilitation was also made more difficult by the fact that there is a great deal of transferring of funds among federal, state, local, and nongovernmental agencies. Not only is there reimbursement from one federal agency to another, funds are also transferred from the federal government to state and local governments and independent organizations as well as from independent organizations to state and local governments. These transfer payments were adjusted in order to obtain a more accurate picture of expenditures. However, it is not certain that information about all of the appropriate transfer payments was available.

Compensation: The second largest amount of expenditures for tuberculosis control goes into compensation, which is comprised mainly of payments to individuals or their relatives because of disability or death caused by tuberculosis. The estimated amount spent on compensation totaled $211 million, an increase of nearly 12 per cent over the figure of $189 million for 1952. This increase is primarily due to more liberal payments to families needing assistance. Since all compensation payments could not be positively identified, the 12 per cent increase is probably a minimum.

Of the estimated total of $211 million spent for compensation in 1956, the federal government's share was $199 million, or about 95 per cent.

State and local government expenditures for compensation consist of estimated funds contributed because of tuberculosis for "aid to dependent children," "aid to totally and permanently disabled," and "general assistance," as well as compensation for the destruction of livestock.

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[2] Hospitals, Journal of American Hospital Association, August 1, 1956, table II, p. 36; August, 1955, Part II, table 12, p. 28; June, 1954, Part II, table 5, p. 26.

There were no reported nongovernmental expenditures for compensation.

Construction: During the past four years, not only have a number of tuberculosis hospitals and sanatoriums been closed, but there has been little construction of new units or expansion of existing facilities. As a result the amount of money spent for construction has declined from $38 million in 1952 to slightly more than $2 million in 1956, a decrease of 95 per cent. Funds spent for the purchase of hospital equipment used primarily for the care and treatment of the tuberculous are also included in this category.

Estimated federal spending accounts for 80 per cent of the total amount spent on construction. Of the $1.6 million spent by the federal government, $763,660 was administered by the Veterans Administration and $827,400 by the Public Health Service under the "Hill-Burton" program.

State and local governments reported an estimated $427,000 spent for hospital construction in 1956. This figure includes funds spent as the state share of "Hill-Burton" construction costs.

Research: The estimates show that $3.9 million was spent on research in 1956, a decrease from the $4.3 million reported in the previous survey. Foundations, pharmaceutical houses, and other donors make grants of money to universities and to other investigators for research. It was not practical to contact all such sources. Those who were queried could not, in most instances, separate the amount spent for tuberculosis research from that spent for other research activities. The $3.9 million estimated for 1956 is, therefore, an underestimate.

The federal government's expenditures for research in 1956 were estimated to be $2.4 million. The Public Health Service's share amounted to $1.5 million, the majority of which was spent on studies of immunization, prophylaxis, and therapeutic agents.

There were no reported expenditures by state and local governments for research in 1956. This does not give an accurate picture, however, as there is a considerable amount of unreported tuberculosis research being conducted in state and local health agencies and hospitals. The cost of this research, however, is usually absorbed in the cost of control programs and/or in hospitalization costs, and is not specifically identified as research expenditure.

The estimated spending by nongovernmental organizations in 1956 was $1.5 million. Nearly 99 per cent of this sum was reported by voluntary health agencies and pharmaceutical research laboratories.

Control: Included under tuberculosis control are funds spent for case finding, public health nursing and laboratory services, control analysis, health education, training programs, administration, and those diagnostic and treatment clinics which are not hospital outpatient clinics. The estimate for 1956 indicates that $58 million was spent for control as compared to the $53 million figure for 1952. This category is definitely underestimated, one reason being that data on expenditures for training of professionals in the tuberculosis field were not available. Another reason is that no data were available on the funds spent for control by universities, other schools, and industry.

The federal expenditures in this area were estimated at nearly $11 million. Of this sum more than $6 million was administered by the Public Health Service mainly in the form of state grants.

More than $29 million was estimated as being spent by state and local governments for tuberculosis control. The figure also includes reported expenditures for the administration of bovine tuberculosis control.

Estimated nongovernmental spending for control was $18 million. The estimate was

taken from reports from voluntary health agencies for such work as case finding, health education, program development, maintenance of clinical and nursing facilities, and administration.

Other activities: Items in this category are taken from estimates from both voluntary health agencies and state and local governments and cover fund-raising activities and tuberculosis control in *other* programs. In 1956 it was estimated that more than $6 million was spent for the various activities included in this category, an increase of more than $3 million over the 1952 figure.

The federal government reports no expenditures in this category.

The estimated expenditures by state and local governments, included for the first time this year, are put at more than $1 million. This covers "supportive services, such as fiscal and personnel activities, centralized purchasing and tuberculosis control services in other programs."[2]

Nongovernmental spending in this category was estimated to be nearly $5 million.

In summary: Although all practicable efforts were made to get an accurate estimate of expenditures for tuberculosis, the figure of $725 million should be considered a minimal amount. This is an increase of more than $100 million since 1952. The largest expenditures were for hospitalization, and it is in this area that the largest increase has been apparent. No attempt was made to estimate the amount of money paid to physicians by nonhospitalized patients, and expenditures in several of the categories shown in this report are known to be understated. Therefore, this estimate of the amount spent because of tuberculosis is conservative.

When considering the cost of tuberculosis to the Nation, it is also important to remember that there are expenses other than cash outlays. The cost to society in the loss of one of its productive members, even though the loss today fortunately is for the most part temporary, cannot be evaluated in dollars and cents. If the estimated value of production lost by persons ill with tuberculosis and the potential future earnings of persons who died with the disease were added, the total would be enormously higher.

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September 23, 1957

[2] Total expenditures for tuberculosis control for fiscal year 1955 and amounts budgeted for fiscal year 1956; State Grants, Review and Accounts, 1956. Department of Health, Education, and Welfare Publications (mimeographed).

SENSITIZATION OF CATTLE ERYTHROCYTES WITH JOHNIN PPD AND TUBERCULIN PPD

The original Middlebrook-Dubos hemagglutination test for tuberculosis[1] employed sheep erythrocytes sensitized with an extract of tubercle bacilli. Although other sensitizing agents have been utilized,[2-5] most of the workers who have modified the test and widened its applicability to diagnoses of sensitizations due to other acid-fast organisms have continued to use sheep erythrocytes. Middlebrook[6] showed that human erythrocytes may also be used, regardless of the presence or absence of tuberculosis in man. In all cases tested by the present writers, human erythrocytes could be sensitized with johnin PPD or tuberculin PPD in solution so that they gave clearer agglutination than sheep erythrocytes (unpublished data). The preparation and use of johnin PPD and tuberculin PPD have been described.[7-12]

During the course of investigation on the serologic diagnosis of Johne's disease and tuberculosis, experiments were undertaken to determine whether cattle erythrocytes could be sensitized. Moreover, an attempt was made to determine whether there was any correlation between the ability to sensitize cattle erythrocytes and such factors as the age, breed, and history of exposure to Johne's disease or tuberculosis of the donor animals.

Positive sera were obtained from cattle which had been artificially sensitized with heat-killed *Mycobacterium tuberculosis* or *Mycobacterium paratuberculosis*[12] and had hemagglutination titers above 1:2,000. In this paper these sera are designated "TB-sera" and "J-sera," respectively.

Erythrocytes from individual animals in five groups were collected in modified Alsever's solution.[3] The cells were washed three times, packed, and resuspended in 10 volumes of Eagle's saline.[3] Solutions of johnin PPD and tuberculin PPD were prepared by suspending 1 mg. of PPD per 0.6 ml. of Eagle's saline overnight at room temperature and then centrifuging to remove undissolved particles. Six-tenths of a milliliter of the supernatant solution of PPD was added to 0.2 ml. of the erythrocyte suspension, and the combined materials were incubated for two hours in a water bath at 37°C. The resulting suspension contained 0.02 ml. of packed cells per mg. of PPD. After incubation, the cells were washed three times and then resuspended in 4 ml. of Eagle's saline. Four-tenths of a milliliter of this suspension of sensitized erythrocytes was added to 0.4 ml. of twofold serial dilutions of bovine serum. In this paper the terms "J-ppd" and "TB-ppd" refer to the portions of the purified protein derivatives of johnin or tuberculin which were soluble in Eagle's saline and were adsorbed by sheep erythrocytes.

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Experiment 1: The erythrocytes from 11 grade Jersey steers, 1 grade Holstein cow, and 8 noninfected, one-month-old grade Jersey bull calves were tested. The cow and 2 of the 11 steers were drenched[13] with intestinal scrapings from animals known to have Johne's disease. Of the remaining 9 steers, 3 were vaccinated with heat-killed *M. paratuberculosis*, 4 were vaccinated with heat-killed *M. tuberculosis*, and 2 were not sensitized. The animals that received a drench had a hemagglutination titer of 1:128 and a johnin skin reaction with a 7 mm. increase in skin thickness. The 7 vaccinated animals had increases in skin thickness of 9 to 12 mm. and hemagglutination titers above 1:1,000. The 2 nonsensitized steers and 8 bull calves were negative on the skin and hemagglutination tests. The erythrocytes of the 3 animals that were drenched and one of the bull calves could not be sensitized with J-ppd or TB-ppd so that they would react with J-sera or TB-sera. The erythrocytes of all of the other animals could be fully sensitized to react with J-sera or TB-sera.

From the results of this experiment it appeared that infection with acid-fast organisms might be correlated with an inability to sensitize an animal's erythrocytes; therefore, the following experiments were conducted. Erythrocytes to be used as controls were obtained from the 11 steers and the cow prior to each of the remaining experiments. The erythrocytes from these animals were retested five times over a period of thirteen months without a change in their ability to be sensitized.

Experiment 2: Erythrocytes were obtained from each of 20 mature grade Jersey dairy cows in a herd known to be free of Johne's disease and tuberculosis. In this group there were 7 animals whose erythrocytes could not be sensitized with J-ppd or TB-ppd against J-sera or TB-sera, 3 whose erythrocytes could be sensitized with J-ppd but not with TB-ppd against J-sera or TB-sera, and 10 whose erythrocytes could be completely sensitized with J-ppd or TB-ppd against both J-sera and TB-sera. There were 10 animals in this group whose sera agglutinated sensitized sheep erythrocytes, but none reached a titer of 1:32.

Experiment 3: Erythrocytes from 38 cows in a dairy herd with a history of Johne's disease were tested. There were 23 cows whose erythrocytes could not be sensitized to agglutinate in the presence of J-sera. The erythrocytes of 18 of these 23 would not agglutinate in the presence of TB-sera. There were 16 animals in this herd whose sera agglutinated sensitized sheep erythrocytes, two of which had a hemagglutination titer of 1:32.

Experiment 4: The erythrocytes from 46 milk cows and one bull, in a herd in which tuberculosis was known to exist, were tested. The information concerning this group, presented in table 1, shows the apparently higher correlation between the presence of lesions and the inability of the erythrocytes to become sensitized than that between lesions and positive hemagglutination tests. Also, it shows the complete lack of correlation between breed and ability of erythrocytes to be sensitized. In this particular herd there was a high correlation between positive skin reactions to tuberculin and the presence of lesions.

Experiment 5: Erythrocytes were obtained from a group of 8 eighteen-month-old grade Jersey steers. When first tested, 2 of these steers had erythrocytes which could not be sensitized, whereas the cells from the remaining six could be sensitized. The 2 steers whose erythrocytes could not be sensitized and 2 steers whose cells could be sensitized were inoculated intraperitoneally with living *M. tuberculosis* strain BCG. The remaining 4 steers were inoculated with living *M. paratuberculosis*. Three months later, when the sera of these animals had reached a hemagglutination titer of over 1:2,000, they were again bled and their erythrocytes tested. No change in reaction was observed. Results obtained

[13] Liquid suspension of material given orally by force.

TABLE 1

ABILITY OF BOVINE ERYTHROCYTES TO BECOME SENSITIZED, COMPARED WITH DIAGNOSTIC TESTS AND BREED AND SEX OF CATTLE

	Sensitization of Erythrocytes			
	With Johnin PPD		With Tuberculin PPD	
	Yes	No	Yes	No
Total Number of Animals	28	19	20	27
Diagnostic tests				
Tuberculin skin test				
Positive	14	14	10	18
Negative	14	5	10	9
Post mortem				
Lesions	13	12	10	15
No visible lesions	15	7	10	12
Hemagglutination test using sheep erythrocytes and tuberculin PPD				
Positive	1	1	1	1
Incomplete	9	6	7	8
Negative	18	12	12	18
Hemagglutination test using sheep erythrocytes and johnin PPD				
Positive	6	4	5	5
Incomplete	8	8	6	10
Negative	14	7	9	12
Breed				
Jersey	19	7	12	14
Guernsey	4	3	3	4
Herford	4	4	4	4
Holstein	1	5	1	5
Sex				
Female	27	19	19	27
Male	1	0	1	0

with this last group were not in agreement with the apparent correlation in the first four groups between presence of infection and ability of erythrocytes to be sensitized.

* * *

Data showing the irregularity of ability to sensitize cattle erythrocytes with johnin PPD or tuberculin PPD have been presented. Of 133 animals tested, 66 (49.6 per cent) had erythrocytes which could not be sensitized.

The ability to sensitize an animal's erythrocytes seems to be constant for any given animal, i. e., it does not change with the age or history of infection of the animal. Nevertheless, the ability to sensitize does not seem to be constant for all animals within a group. Calves and adults, males and females infected or noninfected, and all breeds tested had some individual animals within the group with erythrocytes that could be sensitized and and some with erythrocytes that could not be sensitized.

The only conclusion that can be drawn is that cattle erythrocytes generally are not satisfactory in the hemagglutination test for tuberculosis or Johne's disease unless the

particular donor's erythrocytes have been tested previously against known positive serum and found to accept sensitization satisfactorily.

In summary: When the erythrocytes from five groups of animals, including animals with and without infection with acid-fast organisms, of various ages, breeds, and sexes were used in the hemagglutination test, no correlation could be found between any of these conditions and the ability to sensitize their erythrocytes with johnin or tuberculin PPD.

The erythrocytes from approximately 50 per cent of the 133 cattle tested could not be sensitized with johnin PPD or tuberculin PPD.

It is concluded that cattle erythrocytes are not satisfactory for use in hemagglutination tests for Johne's disease or tuberculosis unless the particular donor's cells have been previously tested for ability to accept sensitization.

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July 9, 1957

EFFECT OF DIONOSIL® BRONCHOGRAPHY ON BLOOD IODINE

Previous observations[1] have shown that the administration of organic iodine compounds for bronchography and cholecystography may cause prolonged elevations of blood protein-bound iodine. Hyde and Hyde[2] made forty-one blood protein-bound iodine determinations on 30 patients after Lipiodol® bronchography and found values up to 400 γ per 100 ml. one week later, and an average of 200 γ per 100 ml. one month later. An average of about seventeen months was required for the protein-bound iodine value to reach normal and, in some instances, three and four years. Such a disturbance in blood iodine prevented its use as a diagnostic agent for many months in patients who had Lipiodol bronchography.

In trying to overcome some of the undesirable features of Lipiodol, Tomich, Basil, and Davis[3] investigated the properties of *n*-propyl ester of 3:5 diiodo-4-pyridone-N-acetic acid (Dionosil), whose diethanolamine salt is known as iodopyracet or diodone (Diodrast®).

Dionosil or propyliodone is a white crystalline solid pulverized to form particles of 5 to 15 micra which can be suspended in either aqueous or oily media. Dionosil aqueous is a 50 per cent suspension of propyliodone in a buffered aqueous vehicle containing sodium carboxymethylcellulose, sodium citrate, sodium chloride, polyethylene glycol 600 monooleate, and benzyl alcohol. It contains about 6 gm. of 30 per cent iodine in 20 ml. of the mixture. Dionosil oily is a 60 per cent suspension of propyliodone in peanut oil. It contains about 7 gm. of 34 per cent iodine in 20 ml. of the mixture.

Tomich and co-workers found that intratracheal injection of the Dionosil mixtures produced pulmonary congestion similar to that seen with Lipiodol, but of shorter duration. The sodium carboxymethylcellulose disappeared from the lungs about the same time as the organic iodide, in five days or less. When I^{131} was used in rabbits, they showed a 95 per cent excretion of the propyliodone in the urine in seventy-two hours. The Dionosil that is not expectorated is hydrolyzed in the bronchial tree and eliminated by the kidney as sodium 3:5 diiodo-4-pyridone-N-acetate; there is no free iodine or iodide ion. Lung clearance occurred within a week or ten days.

A number of clinical reports on Dionosil[4-8] have confirmed the satisfactory roentgenographic picture, absence of alveolar filling, and roentgenographic clearance in several days. The aqueous Dionosil was eliminated in four days and the oily Dionosil in seven days. Cummins and Silver[6] resected a lung seven weeks after Dionosil bronchography and found only occasional droplets.

Dionosil bronchography was done on 34 tuberculous patients using about 20 ml. in each case.

The aqueous mixture was used in 23 cases, the oily suspension in 7 cases, and a mixture which was half oily and half aqueous in 4 cases. Films of the chest were made at five hours, twenty-four hours, and forty-eight hours after bronchography, and then at frequent inter-

[1] Means, J. H.: Thyroid and Its Diseases, ed. 2, J. B. Lippincott, Philadelphia, 1948, p. 165–166.

[2] Hyde, L., and Hyde, B.: J. Lab. & Clin. Med., 1949, *34*, 1516.

[3] Tomich, E. G., Basil, B., and Davis, B.: Brit. J. Pharmacol., 1953, *8*, 166.

[4] Don, C.: Brit. J. Radiol., 1952, *25*, 573.

[5] Adler, D. I., and Fainsinger, M. H.: South African M. J., 1953, *26*, 913.

[6] Cummins, C., and Silver, C. P.: Brit. J. Radiol., 1953, *26*, 435.

[7] Norris, C. M., and Stauffer, H. M.: Ann. Otol., Rhin. & Laryng., 1954, *63*, 520.

[8] Nice, C. M. and Azad, M.: Radiology, 1956, *66*, 1.

vals as long as Dionosil was present. Protein-bound iodine determinations were made immediately prior to the bronchography, (after five hours, twenty-four hours, thirty hours, and forty-eight hours) and then at frequent intervals until a normal value was obtained. A total of 229 protein-bound iodine determinations were made.

The technique used to determine the protein-bound iodine concentrations was the Kingsley and Schaffert modification[9] of the method used by Barker, Humphrey and Soley.[10] The normal range is 4 to 8 γ per 100 ml., with 90 per cent of the values between 5 and 7 γ.

Roentgenographic disappearance of Dionosil: Depending upon the degree of cough, the amount expectorated, and the pathology present in the lung, there was a clearance at five hours of an average of 60 per cent of the Dionosil, with a range of from 25 to 95 per cent. This estimate was made crudely by comparing the Dionosil roentgenographic density at five hours with that made at the time of the bronchogram. At twenty-four hours there was an 80 per cent clearance and 6 patients showed only traces of Dionosil. At forty-eight hours, there was a 90 per cent clearance; 5 patients showed no Dionosil and 8 showed only traces. At the end of seven days, of 31 cases adequately followed, 23 patients (74 per cent) showed no dye retention at all, 6 patients had faint traces, and 2 patients still showed 5 per cent and 25 per cent retentions, respectively. There was no difference between the aqueous and oily mixtures.

The 8 patients showing Dionosil at seven days showed clearing rates as follows: one patient had a retention of 5 per cent in two weeks; 5 patients had traces at two months; one had 20 per cent retention at three months; and one with a trace still showed a faint trace at five months, even though the protein-bound iodine was normal at two months.

Thus, Dionosil begins to disappear very quickly. In seven days, 74 per cent of the patients showed no roentgenographically detectable dionosil, and the rest had only small amounts. In three months, the roentgenograms of all of the patients but one (3 per cent) had returned to normal.

Serum protein-bound iodine values: Immediately prior to bronchography 4 patients had protein-bound iodine values ranging from 8.5 to 11.0 γ per 100 ml. of blood; the others have values ranging from 4.4 to 8.3 γ per 100 ml., with an average of 6.0 γ. Following bronchography, the peak concentrations occurred in 70 per cent at five hours, and in the remainder at twenty-four hours and forty-eight hours. The highest concentration (24 γ per 100 ml. at five hours), was obtained in one case.

The average protein-bound iodine values were as follows: 13.8 γ per 100 ml. at five hours; 12.0 γ at twenty-four hours; 11.1 γ at forty-eight hours; 9.5 γ at five days; 9.1 γ at six days; 8.9 γ at seven days. Normal protein-bound iodine concentrations were attained in one week by 36 per cent of the patients; in one month by 48 per cent more; and in one to five months by the remaining 16 per cent. The pattern of the iodine values showed a rapid initial rise for the first twenty-four hours and then a gradual descent, reaching normal values in the times described. In 8 patients (24 per cent) the usual peak and drop occurred, followed by a mild secondary rise after five or six days which lasted about two days; one patient showed a tertiary rise.

Roentgenographic clearance and return of protein-bound iodine values to normal: Both the roentgenogram and the values for protein-bound iodine returned to normal within a week of each other in 50 per cent of the patients; and in 12 per cent the iodine value became normal while the roentgenogram still showed some Dionosil. In the others the

[9] KINGSLEY, G. R.: Reported in Standard Methods of Clinical Chemistry, vol. II, Academic Press, New York (in press).

[10] BARKER, S. B., HUMPHREY, M. J., AND SOLEY, M. H.: J. Clin. Investigation, 1951, *30*, 55.

values for protein-bound iodine became normal after the roentgenograms; half of them within a month, 16 per cent more within three months.

In summary: Dionosil used bronchographically showed a much more rapid clearing roentgenographically and a much faster return of the protein-bound iodine to normal values than that recorded for Lipiodol. Both aqueous and oily Dionosil disappeared in 70 per cent of the patients in seven days, and in almost all of the patients in three months. The protein-bound iodine values became normal in 84 per cent of the patients in one month and in the remainder within five months. Both indices became normal within a week of each other in 50 per cent of the patients, and within 3 months in almost all cases. It may be advisable to do a routine protein-bound iodine determination one month after roentgenographic clearance following Dionosil bronchography to determine whether a normal value is present.

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July 16, 1957

SERUM CONCENTRATION STUDIES WITH SUSTAINED-ACTION PARA-AMINOSALICYLIC ACID TABLETS

The search for a form of para-aminosalicylic acid (PAS) which will give a low incidence of gastrointestinal reactions and at the same time be satisfactory therapeutically continues. After experimenting with a variety of PAS derivatives, these writers found the following three to be the most generally useful: (*1*) sodium PAS solution; (*2*) buffered PAS tablets;[1] and (*3*) a new form of PAS recently made available to us, sustained-action PAS tablets.[2] Previous trials with various calcium and sodium PAS granules yielded discouraging results with regard to gastrointestinal acceptance. Following a favorable report on the use of the potassium salt of PAS,[3] this form of PAS was tested in a controlled study in four Veterans Administration hospitals[4] with results essentially no different from those obtained with the more commonly used sodium PAS. However, an occasional patient is encountered who can tolerate only potassium PAS capsules. A limited experience with PAS resin-complex (Rezipas®) has borne out a previous report[5] of excellent gastrointestinal acceptance of this preparation. Clinically, however, it is an expensive preparation for routine hospital or sanatorium use.

When sustained-action PAS tablets were made available to this hospital, a preliminary study of patient acceptance of the product indicated that a substantial number of patients who had difficulty with sodium PAS solution and buffered PAS tablets could tolerate this drug satisfactorily. It then became important to ascertain if this form of PAS yielded satisfactory blood concentrations or if, as in the case of some delayed-action tablets previously introduced, it owed its lack of gastrointestinal irritation to unsatisfactory absorption from the gastrointestinal tract.

Patients who were receiving sodium PAS solution or buffered PAS tablets were therefore studied by determining the serum content of PAS four hours after the first dose in the morning, while taking their usual preparation, and after switching to sustained-action PAS tablets. A number of the patients utilized in these studies spontaneously asked if they could continue with this new form of PAS, bearing out a previous experience of good patient acceptance of this product in a substantial number of patients who had had difficulty with sodium PAS solution or buffered tablets. As the primary interest at this time was to determine satisfactory adsorbability of the sustained action product, serum concentrations of PAS were not determined except at the fourth hour after the initial morning dose. This does not imply, however, that the peak serum concentration necessarily occurs at the fourth hour. As a matter of fact, it is likely that much higher serum concentrations are reached after the administration of the second and third doses of PAS which normally occurs at the fifth and tenth hours after the first morning dose on the usual hospital regimen.

The procedure used to determine blood PAS concentrations was a technique, modified

[1] Buffered PAS tablets, from The Panray Corp., are supplied in a 0.5 gm. tablet and represent a combination of PAS and Glycine.

[2] Sustained-action PAS tablets, from The Panray Corp., are supplied in a 1 gm. coated tablet of a fairly large size which some patients find difficult to swallow, and consist of a central 0.5 gm. of free PAS separated from a peripheral 0.5 gm. of buffered PAS.

[3] Cohen, R. V. Molthan, L., and Zarafonetis, C. J. D.: Am. Rev. Tuberc., 1955, *71*, 320.

[4] Myers, W. E.: Tr. Fourteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1955, p. 311.

[5] Hollander, A. D.: Am. Rev. Tuberc., 1955, *72*, 548.

TABLE 1

SERUM VALUE OF PAS AT FOURTH HOUR

	Sodium PAS Solution	Sustained-action PAS Tablets
Number of patients	18	19
Range (mg. per 100 ml.)	2.1–8.3	1.3–10.2
Mean (mg. per 100 ml.)	4.3 ± 1.6	5.4 ± 2.1
Standard deviation	2.00	2.44
P	0.16	

TABLE 2

EFFECT OF CHANGING FROM SODIUM PAS SOLUTION TO SUSTAINED-ACTION PAS TABLETS ON SERUM VALUE OF PAS

Total Patients 17	Per Cent Change	Average Change
		per cent
Increased 11	27–264	75
Decreased 5	10–55	34
No change 1	—	—

in this laboratory, based on that of Way[6] in which the purplish-red dye formed by the reaction of diazotized PAS with N-(1 naphthyl) ethylene diamine dihydrochloride was measured in the Coleman spectrophotometer.

The modified procedure required a 1:20 blood filtrate which was prepared by adding 1 ml. of serum to 15 ml. of distilled water. After thorough mixing, 4 ml. of 15 per cent trichloracetic acid were added, shaken, and allowed to stand for twenty minutes, after which time the precipitated mixture was filtered through No. 42 Whatman filter paper. From this point the remainder of the test was carried out in an ice bath. To 3 ml. of clear filtrate was added 0.5 ml. of 2.4 N HCl. After mixing, 1 ml. of 0.1 per cent sodium nitrite was added, mixed, and allowed to stand for five minutes. Then 1 ml. of 0.5 per cent ammonium sulfamate in 50 per cent acetic acid was added and mixed. This was followed by the addition of 1 ml. of freshly prepared 0.1 per cent aqueous N-(1 naphthyl) ethylene diamine dihydrochloride. After mixing, the solution was allowed to stand for thirty minutes. Finally, 2.0 ml. of distilled water were added and after complete mixing the purplish-red color intensity, which was proportional to the concentration of PAS, was read at a wave length of 540 μ, and compared with a previously plotted standard PAS graph.

The results of substituting sustained-action PAS tablets in equivalent dosage in patients who had been taking sodium PAS solution may be seen in table 1. These data show that, while the range of blood PAS concentration varies widely in both groups, as has been reported frequently in other studies,[7-11] the mean blood concentration was slightly, although not significantly, greater with sustained-action PAS tablets than with sodium PAS solution. Of even greater significance was the result obtained in individual patients on

[6] WAY, E. L.: J. Pharmacol. & Exper. Therap., 1948, *93*, 368.
[7] RISKA, S.: Acta tuberc. Scandinav., 1954, *30*, 144.
[8] DEEB, E. N. AND VITAGLIANO, V. R.: Am. Rev. Tuberc., 1955, *72*, 543.
[9] MILTON, H.: Tubercle, 1953, *34*, 100.
[10] LEHMANN, J.: Svenska LäkT., 1946, *43*, 2029.
[11] VALLENTIN, G.: Svenska LäkT., 1946, *43*, 2047.

substituting sustained-action PAS tablets for sodium PAS solutions. These results are summarized in table 2 and show that of 17 patients whose medication was changed from sodium PAS solution to sustained-action PAS tablets, 11 showed an increase in the serum content of PAS ranging from 27 to 264 per cent, with an average increase of 75 per cent. Only 5 of the 17 patients showed a decrease in serum PAS content, and the magnitude of this decrease ranged from 10 to 55 per cent, with an average decrease of 34 per cent. In one patient there was no change.

The results of substituting a comparable dosage of sustained-action PAS tablets to patients who had been taking buffered PAS tablets are shown in table 3. These results indicate that, while the range of serum PAS values at four hours after ingestion varied widely as in the experiment above, the mean four-hour values obtained with buffered PAS tablets were significantly higher than those obtained with sustained-action PAS tablets. The close agreement in this study both in the range and the mean of the serum PAS values in the 14 patients who received sustained-action PAS tablets with the serum PAS values in the 19 patients in the group in table 1 is emphasized.

Again, apparently because of the wide variation in PAS concentrations which occurred in a group of persons given any form of PAS, the results of changing from buffered PAS tablets to sustained-action PAS tablets can be better evaluated in the individual patient. These results are summarized in table 4, and show that 9 of 17 patients studied showed a decrease in the serum content ranging from 8 to 138 per cent, with an average decrease of 70 per cent when sustained-action PAS was substituted for buffered PAS tablets. Only 2 patients showed an increase of a probably insignificant degree, 6 to 13 per cent, and one showed no change.

Previous studies have indicated that 2 mg. per 100 ml. is an adequate therapeutic concentration of PAS, and it has been suggested that serum concentrations should be 2 to 7 mg. per 100 ml. in humans.[5] These results indicate that sustained-action PAS tablets are satisfactorily absorbed from the gastrointestinal tract and give a satisfactory serum content of PAS. This value at the fourth hour after the ingestion of the first dose in the morn-

TABLE 3

SERUM VALUE OF PAS AT FOURTH HOUR

	Buffered PAS Tablets	Sustained-action PAS Tablets
Number of patients	12	14
Range (mg. per 100 ml.)	4.6–14	2–10
Mean (mg. per 100 ml.)	8.2 ± 1.99	5.6 ± 1.48
Standard deviation	2.5	2.0
p	0.01	

TABLE 4

EFFECT OF CHANGING FROM BUFFERED PAS TABLETS TO SUSTAINED-ACTION PAS TABLETS ON SERUM VALUE OF PAS

Total Patients 12	Per Cent Change	Average Change
		per cent
Decreased 9	8–138	70
Increased 2	6–13	9.5
No change 1	—	—

TABLE 5

EFFECT OF DRUG GIVEN WITH PAS ON SERUM PAS VALUE

Mg. of PAS per 100 Ml. of Serum

	Sodium PAS Solution	Sustained-action PAS Tablets	Buffered PAS Tablets
Isoniazid	5.0	5.4	7.3
Streptomycin	2.9	5.1	9.1

ing tends to be somewhat higher in any individual patient than the value obtained in the same patient with sodium PAS solution in equivalent dosage, but it is significantly lower than the value obtained with buffered PAS tablets.

It has been shown by Riska,[7] who did one thousand PAS determinations in 44 patients with five different PAS preparations, and Deeb and Vitagliano,[8] that PAS serum concentrations are higher following multiple dosage with PAS than after a single dose. On this basis, it seemed logical that a more even PAS serum concentration could be achieved by giving the drug at evenly spaced intervals throughout the twenty-four-hour period rather than on the usual schedule of the hospital, three times daily, at approximately four-hour intervals during the daytime hours. On this supposition, an eight-hour regimen has been followed in a few patients who still had difficulty with sustained-action PAS tablets given three times daily. In several of the patients in whom this maneuver was utilized, it was successful. It should be pointed out, however, that it is not known whether it is more desirable to have a fairly sustained serum concentration of PAS throughout the twenty-four-hour period or whether higher peak concentrations with lower "troughs" may not be just as effective, or even more effective, therapeutically. In this regard, Carr[12] has recently pointed out that a single dose of PAS given every twenty-four hours was more effective therapeutically in the experimental animal than a much larger total dose distributed through the twenty-four hour period. He also found that some patients were better able to tolerate PAS given in a single total dose every twenty-four hours than by the customary administration in three or four doses.

As it has been reported[13] that PAS raises the serum concentration of isoniazid, the present data were analyzed to ascertain if the drug given with PAS had any influence on the serum PAS concentration. These data are summarized in table 5 and indicate that, when sodium PAS solution is given with daily isoniazid, the serum concentration at the fourth hour appears to be significantly higher than when sodium PAS solution is given with 1 gm. of streptomycin twice a week. This difference does not appear to be present when sustained-action PAS tablets are given with daily isoniazid or streptomycin twice a week. In this case, the average concentration is equivalent to that obtained with sodium PAS solution and daily isoniazid. When buffered PAS tablets were given with daily isoniazid and biweekly streptomycin, the difference noted with sodium PAS solutions was not apparent, although as shown in a previous table, the average serum PAS concentration was considerably higher.

In summary: Sustained-action PAS tablets are tolerated by a substantial number of

[12] Discussion at Sixteenth Conference on the Chemotherapy of Tuberculosis, Veternsa Administration, Army, and Navy, 1957.

[13] MORSE, W. C., CURRY, F. J., MORSE, P. D., CHAMBERS, J. S., AND LINCOLN, A. F.: Tr. Fifteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1956,. p 283.

patients who have gastrointestinal disturbances with sodium PAS solution or buffered PAS tablets. They are satisfactorily absorbed from the gastrointestinal tract and give a satisfactory serum concentration of PAS. This value at the fourth hour after the ingestion of the first dose in the morning tends to be somewhat higher in any individual patient than the value obtained in the same patient with sodium PAS solution in equivalent dosage, but it is significantly lower than the value obtained with buffered PAS tablets.

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July 25, 1957

[14] Statistical calculations were made by Mr. Sol Abramowitz, Biochemist, Cardio-Pulmonary Laboratory, to whom sincere thanks are expressed.

Books

DIAGNOSTICS PNEUMOLOGIQUES. By A. P. Jarniou. Pp. 148, G. Doin & Cie., Paris, 1956. Price, 1,350 fr.

This book was designed by the author to be used as a schematic aid for students and practitioners in the study of the diagnosis of pulmonary tuberculosis. As stated in the preface, it could have been titled "Etiologic Diagnosis from Radiologic Syndrome in Respiratory Pathology." In many categories of thoracic roentgenographic syndromes, Professor Jarniou attempts to organize in logical form the anatomic, physiopathologic, and etiologic phenomena which could be responsible for the chosen roentgenographic pattern. In some of the chapters, prognosis and therapy are briefly reviewed.

Unfortunately, the original purpose is scarcely followed in some sections, and the necessity of repetitions, especially concerning etiology, makes the book tedious at times. The reader who is not French will probably be discouraged by the large number of abbreviations, some of which are rather puzzling, and also, for semantic reasons, by the nomenclature used in the section on tumors.

A good discussion in each chapter is devoted to the indications for special examinations. The sections on "bullous lesions" contains an interesting historical survey. The large chapter concerned with the thoracic location of reticuloendothelial diseases includes a broad study of the mediastinal and pulmonary pattern in sarcoidosis (Besnier-Boeck-Schaumann's disease). Noticeably absent in this section is any mention of the nosologic group classified by Lichtenstein under the provisory name of histiocytosis-X (Hand-Schuller-Christian, Abt-Letterer-Siwe, eosinophilic granuloma), although a brief allusion to the Letterer-Siwe syndrome is made.

The book contains some excellent pictures characteristic of described disease, among them one of a staphylococcal pneumonia. By design the author elected to omit a bibliography. Accordingly, references are listed in the text and consist of only the name of the author and the year of publication in each case.

As a reference guide, students may find this book of some value.

CLAUDE BOUVIER
New York, New York

TOPOGRAPHISCHE AUSDETEUNG DER BRONCHIEN IM ROENTGENBILD. By Claus Esser. Pp. 210. Georg Thieme, Stuttgart, Germany. Price, $13.80.

Dr. Esser's work represents the most important advance in the anatomy and roentgenographic interpretation of pulmonary structures, as far as German literature is concerned, since the publication of v. Hayek's book on the anatomy of the human lung. The second edition brings up to date and expands the presentation in the earlier publication, which was so enthusiastically received in Europe.

The first portion of the publication is devoted to a thorough and lucid review of the world literature on pulmonary and, more specifically, bronchial anatomy. This review, which is arranged in chronologic order, helps to clarify and explain the confusion which prevailed in the classification of the human bronchial system in the past. Ewart's early work on human bronchial anatomy was largely disregarded, while Aeby's investigations, done on the lungs of mammals, were considered for many years the most reliable approach to the problem. Difficulties were encountered in attempts to apply nomenclature evolved for lower mammals to the fundamentally different branching type of the human bronchial

system. It was not until the mid-twenties that thorough statistical surveys were made in the United States, France, Germany, and England, leading to a dependable classification of human pulmonary bronchi and segments. The difficulties which had to be overcome are reflected, for instance, in the discussion about the existence of the axillary subsegment bronchus of the right upper lobe or the existence of the mediobasal bronchus of the left lower lobe. Once being established, the bronchial classification proved to be a most important tool for describing the system of the pulmonary vessels.

The descriptive part of Dr. Esser's book is arranged according to the international nomenclature accepted in 1950 in Oxford and improved in 1955 in Paris. The description of every single-segment bronchus begins with a short review of the names previously used. The anatomy of the bronchus, its variants, segmental structure, and blood supply are discussed. These introductory remarks are followed by a demonstration of carefully selected roentgenograms, which illustrate the possible and preferred approaches for the examination of the bronchus, using contrast media, tomograms, or both. All subsegmental bronchi and, in most instances, their branches also are described in a like manner. Every roentgenogram is provided with a sketch to illustrate the direction of the beam.

The book concludes with a short discussion of the dynamics of pulmonary development in the light of recent investigation. The form of the lung is largely determined by function, which again is determined by the characteristics of the mammalian developmental history. One of the most important determinants is the erect gait, requiring a special type of respiration and thus shaping the structure of the thorax and of the bronchial system.

Systematic arrangement of the carefully selected material and a thorough and exhaustive discussion of the subject make this book a valuable help for any investigator interested in the clinical or theoretical aspects of the human lung.

DIETHELM BOEHME
New York, New York

BOOKS RECEIVED

BCG & VOLE VACCINATION. Second Edition. By K. Neville Irvine. Pp. 103, National Association for the Prevention of Tuberculosis, London, England, 1957. Price $3.00.

LE PNEUMOPERITOINE AU SANATORIUM. By Daniel Cox and David Gelibter. Pp. 360, L. Van Looveren, Brussels, Belgium.

LES MALFORMATIONS CONGÉNITALES DU POUMON. By André Breton and Oliver Dubois. Pp. 126, plus illustrations, G. Doin & Cie, Paris, France, 1957. Price, 2,200 fr.

HOST-PARASITE RELATIONSHIPS IN LIVING CELLS. Edited by Harriet M. Felton. Pp. 245, Charles C Thomas, Springfield, Illinois, 1957. Price, $6.50.

DAS SOGENANNTE ALVEOLARZELLKARZINOM. By Hermann Eck. Pp. 71, Veb Georg Thieme, Leipzig, Germany, 1957. Price, 14 DM.

PATHOLOGICAL HISTOLOGY. Fifth Edition. By Robertson F. Ogilvie. Pp. 482, The Williams & Wilkins Company, Baltimore, Maryland, 1957. Price, $3.75.

LA RADIOLOGIE PLEURO-PULMONAIRE (SAVOIR INTERPRÉTER). By L. Babaiantz and F. Cardis. Pp. 96, Les Editions De Visscher, Brussels, Belgium, 1956. Price, 75 fr. b.

TESTING AND TEACHING OF PHOTOFLUOROGRAM INTERPRETERS. By Helge Nielsen. Pp. 32, Ejnar Munksgaard, Copenhagen, Denmark, 1957. Price, 9.50 Dan. kr.

HALSTED OF JOHNS HOPKINS. By Samuel James Crowe. Pp. 247, Charles C Thomas, Springfield, Illinois, 1957. Price, $5.00.

SIR ROBERT W. PHILIP (1857–1939). Edited by Harley Williams. Pp. 96, National Association for the Prevention of Tuberculosis, London, England, 1957. Price, 12/6d.

SYMPOSIUM OF TUBERCULOSIS. Edited by F. R. G. Heaf. Pp. 755, Cassell and Company Limited, London, England, 1957. Price, $20.00.

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NOTICES

The following dates and locations of the next two annual meetings of the National Tuberculosis Association and the American Trudeau Society have been selected and approved:

1958—Philadelphia, Pennsylvania—May 18–23
1959—Chicago, Illinois—May 24–29

THE MEASUREMENT OF PULMONARY FUNCTION IN HEALTH AND DISEASE—POSTGRADUATE COURSE, March 24–28, 1958

A postgraduate course on the Measurement of Pulmonary Function in Health and Disease is sponsored by the medical schools of Harvard University, Tufts University, and Boston University, the Harvard School of Public Health, the Massachusetts Tuberculosis and Health League, and the Massachusetts Trudeau Society.

A course aimed at physicians interested in diseases of the chest who wish to acquaint themselves with the methods used in the evaluation of pulmonary function will be presented for the eighth successive year at the Boston City Hospital, March 24 to 28, 1958. The methods used in the evaluation of pulmonary function and related cardiac function will be demonstrated.

Tuition will be $75.00. Applications and more detailed information may be obtained from the chairman of the course, Edward J. Welch, M.D., 1101 Beacon Street, Brookline 46, Massachusetts.

FORTY-THIRD ANNUAL SESSION OF THE TRUDEAU SCHOOL OF TUBERCULOSIS—1958

The Trudeau School of Tuberculosis will present its Forty-Third Annual Session commencing Monday, June 2, and concluding Friday, June 20. The concentrated three-week course will cover all aspects of pulmonary tuberculosis and also certain phases of other chronic chest diseases, including those of occupational origin.

As in the past, the clinical material for the course will be derived from the Ray Brook State Tuberculosis Hospital, the Tupper Lake Veterans Hospital, Will Rogers Memorial, and Sanatorium Gabriels. The research laboratories of the institutions in the area and the practicing tuberculosis specialists in Saranac Lake will be called upon, as formerly, to participate in the program. There will also be participation by authoritative guest lecturers from different parts of the country.

The schedule for the 1958 course is in preparation. If you wish, a copy will be mailed to you as soon as it is available.

Registration is limited, and it is suggested that those who plan to attend make early application for enrollment.

The tuition is $100, payable to the Trudeau School not later than the opening date, June 2, 1958. A few scholarships are available for those who qualify.

An application form and more detailed information may be obtained by writing to:

Secretary
Trudeau School of Tuberculosis
Box 500
Saranac Lake, New York

FIFTH INTERNATIONAL CONGRESS ON DISEASES OF THE CHEST

The Fifth International Congress on Diseases of the Chest, sponsored by the American College of Chest Physicians, will be held in Tokyo, Japan, September 7–11, 1958. The Congress will be presented under the patronage of the Government of Japan and the Japan Science Council. The Congress has been endorsed by the Japan Medical Association.

For additional information, please write to: Dr. Jo Ono, Secretary General, Fifth International Congress on Diseases of the Chest, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku, Tokyo, Japan; or Mr. Murray Kornfeld, Executor Director, American College of Chest Physicians, 112 East Chestnut Street, Chicago 11, Illinois.

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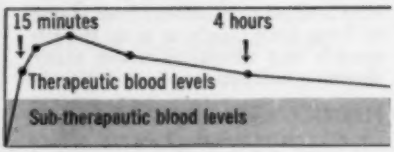

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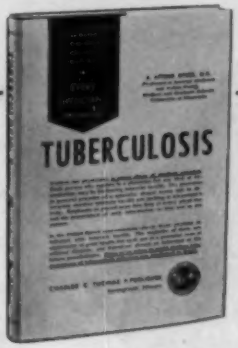

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- Discusses the abandonment of several practices and teachings which have become useless because of changed situations in some parts of the world and others which have never been useful but are still employed.

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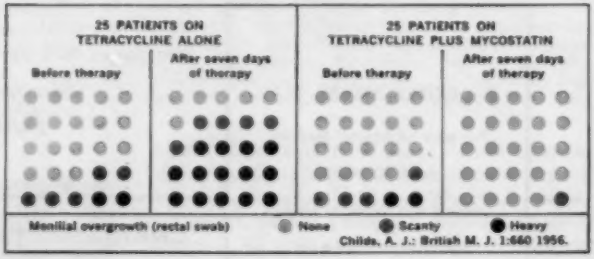

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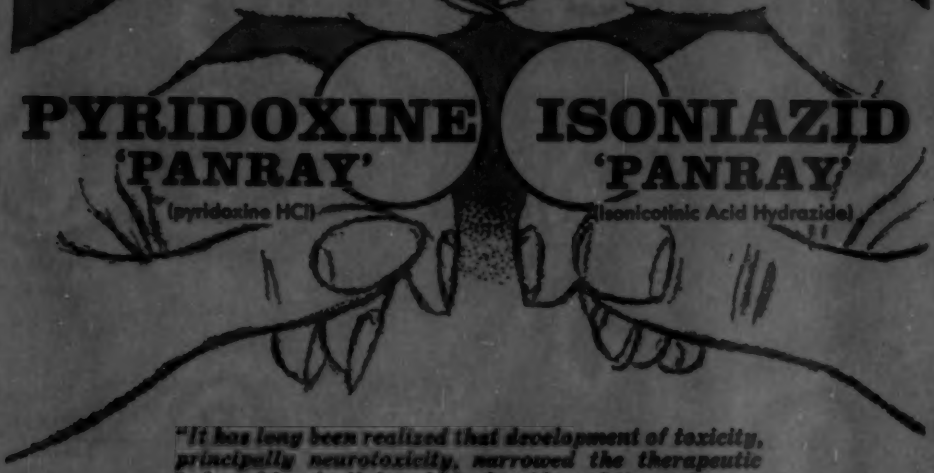

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... pyridoxine is an effective prophylaxic agent when large doses of INH are given and that when neurotoxic signs develop pyridoxine hastens recovery."[1]

Concomitant regimen of B_6 and INH assures uninterrupted chemotherapy by avoiding neurotoxicity.

Pyridoxine 'Panray' does not interfere with the bacteriostatic action of INH.

Pyridoxine 'Panray' — 10 mg., 25 mg., 50 mg. and 100 mg. tablets.

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